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Cytological Studies Of Metaplasia

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CYTOLOGICAL STUDIES

OF

METAPLASIA

by

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ABSTRACT

Metaplastic transformation of epithelial and connective tissue cells has been studied in various tissues by means of electron microscopy.

Metaplasia of tracheal and urinary bladder epithelium induced by vitamin A deficiency commenced with localized areas of hyperplasia of basal cells, which later spread and undercut the original, but somewhat modified, superficial cells. In the trachea these superficial cells showed a loss of some of the specific features of tracheal epithelial cells; in the urinary bladder they showed some of the features of keratinizing cells. In both cases, however, the fully cornified (epidermoid) cells differentiated from the clones of hyperplastic basal cells.

The significance of membrane-coating granules and keratohyalin granules in the process of epidermoid change was studied. Both types of granules were observed in epidermoid metaplasia (as they are in normal epidermis). The peculiar vesicles of bladder epithelium, the fusiform vesicles, which have asymmetrical unit membrane, were observed to take on certain features of membrane-coating granules during early stages of metaplasia.

Langerhans cells, usually considered to invade the epidermis when they migrate out from the neural crests, were found to appear in areas of epidermoid metaplasia. Obviously, this finding is inconsistent with the accepted origin of these cells, and suggests that they

are derived from the mesenchymal cells.

Attempts were made to study the metaplastic change from squamous to mucous secreting epithelium by application of excess of vitamin A to the hamster cheek pouch and to cultures of chick embryo skin. Evidence was obtained in both studies of at least the relative suppression of keratin formation, and in the hamster cheek pouch of the additional initiation of mucous synthesis and eventual mucous metaplasia.

Mucous metaplasia was further studied in the vagina of castrated mice subjected to hormone treatment. The separate effects of stimulation of mucus production by progesterone and stimulation of keratinization by estrogen were observed. The co-existence of the capacity for mucous and keratin synthesis in an individual cell was observed.

Cartilaginous metaplasia, studied in the regenerating tendon of rats, was observed to show several differences from normal chondrogenesis. These were: the cells showed more cytofilaments; the matrix was scanty; matrix granules were few; collagen fibrils were abundant.

Most of the metaplastic sites first appeared at a time when reinforcement for the regenerating tendon was needed i.e. two or three weeks after tenotomy.

A peculiar feature of all types of cells subjected to the conditions of these experiments was the frequent observation of single cilia among them. This is believed to be a common expression of cells subjected to adverse conditions of growth.

I. INTRODUCTION

Metaplasia is the formation of one type of adult tissue by cells that normally produce tissue of another type. The metaplastic tissue is epithelial if the parent cells were epithelial, and is a form of connective tissue if the parent cells were of that origin. A very wide variety of metaplasia may exist in the various tissues. Sometimes there is no significant effects on the normal function of the tissue or organ; many metaplasias are related to physical conditions, nutritional conditions, or pathological conditions, including neoplasm.

The epithelial metaplasias are of two types: (a) squamous or epidermoid or (b) glandular or mucous.

Squamous or epidermoid metaplasia is the transformation of glandular or mucosal epithelium into stratified squamous epithelium, while glandular metaplasia is the transformation of non-glandular tissue into mucous secreting cells, or of a gland of one type into another type. There is an enormous literature on metaplasia of the squamous type and a lesser amount on the glandular type in human pathology. Both types may be associated with either non-neoplastic or neoplastic conditions (Willis, 1958).

The experimental studies are relatively fewer. The effect of vitamin A deficiency, which produces extensive squamous metaplasia of respiratory, urinary, and other epithelial surfaces, was discovered by Wolbach and Howe (1925). The effect of estrogens in producing mucous metaplasia in the vaginal epithelium of castrated mice has also been

described (Meyer and Allen, 1933; Macdonald and Ronson, 1939). Mucous metaplasia has also been induced by hypervitaminosis A, both in vitro (Fell and Mellanby, 1953; Hardy, 1967, 1968) and in vivo (Lawrence, Bern and Steadman, 1960). However, all of these studies were made at the light microscopic level, and the details of the fine, sequential, structural changes during the transformation are not known.

On the subject of connective tissue metaplasia, there exists a large literature dealing with the formation of cartilage or bone in scars, in haemorrhagic areas, in inflammatory and degenerative lesions (Greiz, 1933; Willis, 1958). Cartilaginous and bony metaplasia can readily be induced by transection of the Achilles tendon of the rat (Buck, 1953), by auto- or homo-transplantation of the urinary bladder mucosa to fascias or to subcutaneous tissue (Huggins, 1931a; Anspach, 1964; Mestel and Spain, 1967), or by injection of human amniotic cells of FL line (Anderson, Marker and Fogh, 1964; Anderson, 1967). However, except for one (Anderson, 1967) all these studies were done at the light microscopic level.

These problems remain: (1) Do metaplastic tissues originate from the already differentiated cells or from relatively undifferentiated ones? (2) Does the metaplastic change represent an all-or-none reaction or does it exist in transitional forms? (3) If the latter is the case, what are the sequential steps taken by this change? (4) What structural resemblances do the metaplastic tissues bear to the parental tissue? (6) What are the factors that control the metaplasia, and how stable is the metaplastic state? (7) Is there any structural difference between metaplastic squamous epithelium and benign tumours of the epithelial surfaces? (8) Is squamous or glandular metaplasia a precancerous state?

The present knowledge in these areas gives no firm answer to some of these questions. It was strongly felt that a more systematic investigation on various aspects of metaplasia was needed. The present investigation was divided into four sections.

The first section deals with squamous metaplasia of mucous surfaces. Trachea and urinary bladder were selected because they represent different types of epithelial surfaces which under normal conditions have very different morphology and functions (Rhodin and Dalhamn, 1956; Rhodin, 1959; Hicks, 1965). However, when the animal is subjected to vitamin A deficiency (Wolbach and Howe, 1925) both of these tissues undergo squamous metaplasia. It is of interest to observe and compare the changes in these epithelial surfaces in vitamin A deficiency.

The second section deals with the glandular or mucous metaplasia of squamous (or potentially cornified) epithelial surfaces. These were represented by hamster cheek pouch epithelium and the chick embryo skin. The former was studied in vivo, and the latter in vitro, in the presence of excess vitamin A. It was hoped that through these experiments a better insight into mucous metaplasia would be provided.

The third series of experiments was designed to study the hormonal factors that influence the mucous metaplasia of vaginal epithelium of ovariectomized mice, and to compare this metaplasia with that obtained by hypervitamin A in the hamster cheek pouch.

The fourth group of experiments was concerned with cartilaginous and bony metaplasia in regenerating tendon, and the stability of these metaplastic tissues when transplanted to subcutaneous tissue.

Langerhans cells, which are normally found in epidermis, were observed in metaplastic tracheal and urinary bladder epithelia of vitamin A deficient rats. This can be considered as single cell metaplasia (Järvi, 1962), the observations on which will also be included.

Finally, cilia were observed in cells which do not usually have them. The frequency of their presence warrants special attention.

II. HISTORICAL REVIEW

Metaplasia is a condition not so well-known to biologists as to pathologists for the obvious reason that metaplasia is an abnormal transformation quite often seen in pathological histology. Metaplasia has been observed in almost all the tissues of the body (Willis, 1958). Many cases are associated with neoplastic lesions, while others have relation to other types of disease. It is not the intention in this thesis to review all the literature on metaplasia, but to restrict the survey to those closely related to the present investigation.

1. Squamous Metaplasia

(a) Metaplasia in tracheal and bronchial epithelium.

(b) Association with carcinoma.

There is the question of the significance of squamous metaplasia as a stage in the development of malignant neoplasms of the respiratory tract (Spain, 1959; Auerbach, Stout, Hammond and Garfinkel, 1961). The reason for this is the close association of squamous metaplasia with tracheo-bronchial carcinomas (Valentine, 1957). Valentine (1957) reported that in 144 autopsied patients, dying of "noncancerous disease", the average incidence of squamous metaplasia of the bronchus was 36.8 percent while the incidence in 23 patients with bronchogenic carcinoma was 56.5 percent. Lindberg, in 1935, reported 15 cases of modified, chiefly metaplastic, epithelium in 39 cases of bronchial carcinoma. It was associated commonly with squamous cell carcinoma and was not found in adeno-

carcinoma. Black and Ackerman (1952) found squamous metaplasia in all but seven of their 60 cases (88 percent) of epidermoid and undifferentiated carcinoma of the bronchus. Weller (1953), from a study of large and small bronchi among 128 autopsies, found a 32 percent incidence of metaplastic epithelium, but five of his seven cases of primary lung cancer showed metaplasia.

Some authors reported that epidermoid carcinoma in situ of bronchus arises from or is associated with areas of squamous metaplasia (Papanicoloau and Koprowska, 1951; Umiker and Storey, 1952; and Williams, 1952). In fact, squamous metaplasia in the lung is considered by many to be a precancerous stage (Womack and Graham, 1942; Reingold, Ottoman and Konwaler, 1950; Berkheiser, 1959; Rigdon, 1959; Rockey, Speer, Ann, Thompson and Hirne, 1962). Evidence suggesting a morphologic sequence leading through squamous metaplasia to epidermoid carcinoma of the respiratory tract has been put forward (Reingold et al., 1950; Black and Ackerman, 1952; Della Porta, Kalb and Shubik, 1958; Weller, 1953 and Rockey et al., 1962).

Squamous metaplasia has also been produced in animals following the introduction of carcinogens. Dibenzanthracene, which produces lung tumours in rats, often produces an accompanying epithelial metaplasia (Niskanen, 1949).

On the other hand, there have been numerous reports which showed that squamous metaplasia is of no importance in the pathogenesis of carcinoma in trachea and bronchus (Niskanen, 1949; Auerbach et al., 1961; Auerbach, Stout, Hammond and Garfinkel, 1962a; and Spain, 1959). Niskanen reported a 2.5 percent incidence of squamous metaplasia among 78 cases of carcinoma of the bronchus but a 60 percent incidence among chronic inflammatory disease. Valentine (1957) observed an incidence of metaplasia of 61.9 percent in 21 patients with non-cancerous pulmonary disease.

The experience of most observers has been that such alteration as basal cell hyperplasia, stratification and squamous metaplasia are not associated with any special predilection for the development of bronchogenic carcinoma. It is true that squamous metaplasia (epidermatization) is quite common in tracheobronchial carcinoma, but in most of the cases it is considered to be a secondary effect of the tumour rather than its precursor. No conclusive answer can be drawn as to whether or not metaplasia is precancerous. Adding to the confusion is the fact that no solid criteria for squamous metaplasia are agreed upon. This term has been used to refer to epithelia of squamous (or just multilayered) nature, either with or without cornification or with or without the association of cellular atypias. Švejda and Dolezel (1964) and Feit, Švejda and Hoffman (1967) are of the opinion that only metaplasia with a marked proliferation of basal cells and with cellular atypias may be regarded as precancerous.

According to Spain (1959) the recognition and proper interpretation of early alteration in the bronchial epithial lining are of the utmost importance in the evaluation of the significance of various factors in the causation of bronchogenic carcinoma. In his opinion, a distinction should be made, as it has not been made in many reports (Auerbach et al., 1957; Valentine, 1957), between regenerative lesions that do not have the biologic potential for malignant neoplasia and those atypical alterations that have an enhanced potential.

(ii) Squamous metaplasia in chronic diseases.

It is well known that squamous metaplasia is found commonly in chronic pulmonary diseases (Teutschlaender, 1919; Nicholson, 1917; Niskanen, 1949; Valentine, 1957). Niskanen (1949) reported that 60 percent of

patients with inflammatory diseases, such as chronic pneumonia, bronchiectasis, chronic bronchitis, or pulmonary tuberculosis showed prominent squamous metaplasia. Valentine (1957) also reported the presence of metaplasia in more than half of his 21 patients who died of bronchial asthma, pulmonary emphysema, bronchiectasis, chronic pulmonary tuberculosis or silicosis. Nicholson (1922a), Straub and Mulder (1948) and Auerbach, Petrick, Stout, Statsinger, Muehasm, Forman and Gere (1956) observed squamous metaplasia in patients with chronic bronchopneumonia, influenzal pneumonia and bronchitis. As mentioned above, the high incidence of squamous metaplasia in these diseases tends to complicate the question of whether or not squamous metaplasia is precancerous.

(iii) Irritants.

A large number of irritants can cause squamous metaplasia. They can be chemical, mechanical or thermal. Auerbach et al. (1957, 1961, 1962b) reported that there is a direct relation between the extent of squamous metaplasia and the amount and duration of cigarette smoking. It was found less frequently in nonsmokers with chronic inflammatory diseases than in cigarette smokers (Auerbach et al., 1962b; Weller, 1953). Chang (1957) reported the more frequent occurrence of hyperplastic and metaplastic changes in the lungs of smokers as compared with the lungs of non-smokers.

Tobacco tar, produced in a smoking machine, applied (0.05 to 0.1 mg.) topically to the bronchial mucosa of dogs by bronchoscopy, effectively produced squamous metaplasia (Rockey, Kuschner, Kosak and Meyer, 1958). Intermediate stages of metaplasia were observed in dogs after only three days during which they received only two treatments. Furthermore, squamous metaplasia was consistenly found in animals treated with tobacco tar for 17 days or more. The control group that had undergone more than 100 "sham"

application procedures showed only mild hyperplasia. Tipton and Crocker (1964) also reported that squamous metaplasia appeared in biopsies of bronchi of dogs only three days after smoke condensate application. They also showed that cigarette condensate had a long-acting effect on bronchial mucosa. Animals were sacrificed at various intervals up to 18 weeks after a period of treatment. Squamous metaplasia was very prominent a few days after the last treatment. Although the incidence of frank metaplasia decreased five weeks after treatment, the epithelium did not return completely to normal morphology until the 18th week. Leuchtenberger, Doolon and Leuchtenberger (1958) reported successful production of hyperplasia and metaplasia in bronchi of mice exposed to tobacco smoke for 200 days. On the other hand, dogs exposed to cigarette smoke in smoking machines failed to develop complete squamous metaplasia, as observed with either the light microscope (Rockey and Speer, 1966; Auerbach, Hammon, Kirhian, Garfinkel and Stout, 1967), or electron microscope (Frasca, Auerbach, Parks and Jamieson, 1968).

(iv) Healing and squamous metaplasia.

Nasatracheal intubation, commonly used to assist infants and children with respiratory inadequacy, has been shown to induce metaplasia of tracheal mucosa (Symchych and Cadatte, 1967). Similar observations have been reported by Teplitz, Epstein, Rose and Moncrief (1964) and Striker, Stool and Downes (1967). They believed that nasatracheal intubation created some mechanical disruption of the mucosa which healed poorly in the presence of the nasatracheal tube and normal epithelium was replaced by squamous cornified epithelium. Burian (1960) studied the results of extensive and deep damage to the nasal mucosa of rats. The resulting denuded areas healed and were covered by irregular sheets of squamous epithelium. This type of epithelium was replaced by normal

epithelium in eight to ten weeks. When rat tracheal epithelium was curetted the wound was quickly re-epithelialized by simple stratified epithelium, but this soon differentiated into ciliated respiratory epithelium (Wilhelm, 1953).

(v) Dietary conditions and squamous metaplasia.

The effect of vitamin A deficiency, which produces extensive squamous metaplasia of respiratory epithelium, was first recognized by Mori (1922) and was more systematically studied by Wolbach and Howe (1925, 1933), Wolbach (1937), Tyson and Smith (1929), Seifried (1930) and Niskanen (1949). Wolbach and Howe gave as their opinion that vitamin A deficiency produces an atrophic modification of the original epithelium which in turn initiates a reparative proliferation of the basal cells and the formation of squamous epithelium. Several authors (Tyson and Smith, 1929; Wolbach and Howe, 1925; Wolbach, 1937; Seifried, 1930) showed that the metaplasia produced by vitamin A deficiency was reversible, except in organs where inflammation caused extensive fibrotic changes.

(b) Squamous metaplasia of bladder epithelium.

The phrase, "squamous metaplasia with cornification" (when applied to renal pelvis and bladder), has been called leukoplakia by most clinicians and pathologists. According to Willis (1958), the term leukoplakia is applied to hyper-differentiation of an epithelial surface which is normally stratified squamous. In the renal pelvis and bladder, where the epithelium is transitional type, the term squamous metaplasia is more appropriate.

Squamous metaplasia of the bladder was first mentioned in 1861 by Rokitansky. In 1929 Kutzmann collected 67 cases of renal pelvic metaplasia, and in 1936 Rabson reviewed 127 cases of bladder metaplasia. Since then a number of other reports of metaplasia have been added both of renal

pelvis (Abehouse and Tankin, 1956; Politano, 1956; Falk, 1954; Armstrong, Harlin and Fort, 1950; Madsen, Slotkin and Niesen, 1961; Besemann, 1967) and bladder (Patch, 1948; Abehouse and Tankin, 1956; Hedenberg, 1954; O'Flynn and Mullaney, 1967; Holley and Mellinger, 1961; Gazayerli, 1964; Kelalis, Emmett and DeWeerd, 1963; Thompson and Stein, 1944; Connery, 1953). Some of these lesions were associated with neoplasia; others were related to diseases of chronic nature or to irritants.

The trigonal region of the urinary bladder is the most favourable site for squamous metaplasia. It usually starts at that region and spreads from there over the whole bladder (Arons and van der Rijst, 1932).

Squamous metaplasia of renal pelvis and bladder have long been considered by many clinicians and urologists to be precancerous (O'Flynn and Mullaney, 1967; Thompson and Stein, 1940; Rabson, 1936; Kutzmann, 1938; Holley and Mellinger, 1961). In Rabson's (1936) extensive review of 127 cases, including three which he observed personally, he found a 15 percent incidence of squamous metaplasia associated with carcinoma. Connery (1953), who reviewed 45 cases of metaplasia which had been detected clinically over a period of 34 years, found a 20 percent incidence of associated squamous cell carcinoma. Of the 67 cases reported by Kutzmann (1929) six had associated malignancy of renal pelvis, and two of the bladder. In the 46 cases reviewed by Madsen et al., (1961) six had associated renal pelvis carcinoma. In 1961 Holley and Mellinger reported that 15-20 percent of cases of squamous metaplasia of urinary bladder were associated with carcinoma. Most importantly, they followed up a patient with squamous metaplasia by cystoscopy for several years, and this patient eventually developed carcinoma.

Similar cases have also been described by Kelalis et al. (1963) and O'Flynn and Mullaney (1967). In the latter report biopsies were

taken in addition to cystoscopic examination. The two patients who became carcinomatous, initially and on follow-through, showed very different histological pictures. The first patient had squamous metaplasia for many years and the histologic feature of biopsies was degenerative change. This epithelium later became neoplastic and a rapidly developing carcinoma ensused. A more gradual change was observed in the second case. The lesion was initially more hypertrophic, with marked parakeratosis. Atypical cells with loss of polarity were found one year prior to the onset of frank squamous carcinoma. This finding would be quite compatible with the idea expressed by Rabson (1936), that it is probable that squamous cell carcinoma in the bladder is caused by the same factors responsible for squamous metaplasia, and that the carcinoma represents a "further distortion and exaggeration of the metaplastic process".

Several earlier authors have also reported the transition between the two conditions. Hinman, Kutzman and Gibson (1924) observed, in sections of squamous metaplasia of the renal pelvis, what they thought must be regarded as precancerous lesions. Hallé (1896), in a case in which the metaplasia and squamous carcinoma were associated, found definite intermediate steps between the two conditions. Patch and Rhea (1935), who studied cases of associated metaplasia and carcinoma by serial sections, were able to demonstrate evidence of a transition between the two conditions.

Against this, it has been urged that not all cancers of the urinary tract are preceded by squamous metaplasia. Furthermore, in only a relatively small number of cases are metaplasia and squamous cell carcinoma found in association. Thompson and Stein (1940) reported that only five percent of 34 patients had metaplasia associated with carcinoma in the Mayo Clinic over a period of 25 years. The question is complicated

by the fact that a high incidence of all the reported cases had long standing urinary tract infection (Madsen et al., 1961).

In Schabad's (1959) series of 32 cases, only one did not have pyuria. In Connery's (1953) series of 49, all had accompanying infection. The 20 patients reported by O'Flynn and Mullaney (1967), including the two cases progressing to squamous cell carcinoma mentioned earlier in this review, had chronic diseases of one type or another for years, including ureterectasis, pyelouriterectasis, gonorrhoea or vesical calculi. Metaplasia has also been observed in tuberculosis and venereal disease, but the incidence was rather low (Holley and Mellinger, 1961).

Squamous metaplasia has been induced in the urinary bladder of rats (Wolbach and Howe, 1925; Hedenberg, 1954) and in germ-free rats (Beaver, 1961) by vitamin A deficiency or by treatment of rats with high doses of follicular hormones (cited by Madson et al., 1961). It is of interest to note that metaplasia occured in germ-free animals, without accompanying infection or inflammation, as it had been suggested that squamous metaplasia in vitamin A deficiency might be due to infection. It can also be induced by implanting foreign bodies into the bladder of rats (Capurro, Angrist, Black and Moumgis, 1960; Angrist, Capurro and Moumgis, 1960). Recently, dietary depletion of vitamin A has been used to induce metaplasia in order to study the precise morphological changes by electron microscopy (Ghidoni and Campbell, 1969; Hicks, 1968). It was thus shown that the metaplastic urinary epithelium is structurally similar to the epidermis (Odland, 1958; Matoltsy and Parakkal, 1967). Four strata (basalis, spinosum, granulosum and corneum), found in the epidermis, can be readily identified. There was no indication of invasion of the basement membrane by the metaplastic cells.

As shown by Wolbach and Howe (1925, 1933) and Wolbach (1937), squamous metaplasia can be reversed when a normal diet is given. However, squamous metaplasia developed under conditions other than vitamin A deficiency (in man) is not satisfactorily reversed by various treatments, according to a number of authors (O'Flynn and Mullaney, 1967; Holley and Mellinger, 1961). The reversibility of squamous metaplasia of the urinary tract requires further study.

2. Mucous Metaplasia

(a) Mucous metaplasia related to the concentration of vitamin A in culture.

Fell and Mellanby (1953), studying the direct effect of vitamin A on chick embryo skin in culture, found that vitamin A when present in excess (20-30 i.u./ml.) had the ability to convert chick embryo epidermal cells to mucous secreting cells. Ciliated cells were observed in some cultures exposed to high vitamin concentration. The skin used was seven-day old chick embryo trunk skin, cultured on a plasma clot. In order to find out whether vitamin A had the same effect on older chick embryo skin, Fell (1957) repeated experiments with 13- to 18- day chick skin. In the vitamin A treated (10 i.u./ml.) 13-day skin, mucous metaplasia was observed to be the same as in the seven-day chick skin. However, in the 18-day embryo, although keratinization was regularly suppressed, mucous metaplasia was less regularly observed.

Vitamin A is known to act directly on the epidermal cells, and its effect is not mediated through the dermis. McLoughlin (1961a,b) isolated the epidermis from the dermis by trypsin digestion and cultivated it with or without the addition of vitamin A. Without added vitamin A, squamous epithelial cysts were formed, whereas in the vitamin A

treated explants, cysts lined by mucous secreting cells were found.

Weiss and James (1955) demonstrated that brief exposure to a high concentration of the vitamin A solution was sufficient to produce mucous metaplasia. They, therefore, concluded that vitamin A does not alter the metabolism of the cells gradually during their growth, but acts as an inductive agent which switches on the mechanism for mucous differentiation. However, this idea was refuted by Lasnitzki and Greenberg (1956) who showed that, as vitamin A is retained in the tissue for up to five days after a brief exposure, it could continue to act on the differentiating cells. The 13-day skin responded to a low concentration of vitamin A. Extensive mucous metaplasia was obtained with only 2.5 i.u. vitamin A per ml. of culture medium (Fell and Glover, cited by Fell and Rinaldini, 1965). The attempt of Simon (1967) to induce mucous metaplasia of chick skin in chemically defined medium with excess vitamin A was not successful. Although alcian blue granules were observed, true mucous metaplasia was not obtained.

More recently, Fitton-Jackson and Fell (1963) have examined the fine structure of this type of mucous metaplasia by electron microscopy. They found that after seven days in culture in the presence of excess of vitamin A the superficial layer of epidermal cells had formed many microvilli and mucous granules were present in the cytoplasm.

Although no mucous granules were found in the basal cells in the vitamin treated cultures, keratin fibrils were sparse. In the control cultures, keratin fibrils were prominent both in basal and superficial cell layers. In addition, they found that when these explants were transferred to normal medium for another two days mucous secretion was profound in the superficial layer. In many cells at intermediate levels of the skin, mucous globules, together with keratin fibrils, were

present. This suggests that the same cell can carry on both functions at the same time.

Lasnitzki (1958) applied the same method for cultivating human foetal skin and failed to produce metaplastic change. New (1963) was no more successful with cultures of the dorsal skin of foetal mice and rats. On the other hand, Hardy (1967) using Maximow slides for organ culture, was able to show several changes in 13 1/2- to 15-day mouse embryonic skin with hypervitaminosis. She found that the normal keratinization of mouse skin was inhibited. In addition, some intracytoplasmic alcian blue positive bodies were observed indicating the presence of mucopolysaccharide. New (1965), in a second study, reported similar results for skin of embryonic rabbit food-pads and tail.

Recently Hardy (1968) cultured vibrissal follicles of mouse skin of various ages in solid clots with excess vitamin A. She observed mucous metaplasia in some of the hair follicles. The outer root sheath, or less differentiated follicle cells, formed branching tubular glands. In some cases the entire follicles were transformed to tubular glands. She also observed that follicles of different regions of the skin of the same embryo reacted quite differently, because of the lack of uniformity in the time of hair follicle development at different regions of the body. She thought that there might be a critical period in follicle development for the metaplastic action of vitamin A to have effect. The majority of the mystacial follicles from 14-day embryos reached stage 3c or 4 of development (Hardy, 1949; Hardy, 1967) at the critical time (at 17 or 18 days, three or four days in culture) and thus metaplasia was widespread. The submentel follicles from 14-day embryos reached stage 4 at about 20 or 21 day (six or seven days in culture) missed the critical time (which is three or four days in culture) and did not undergo

metaplasia. Whether or not these findings are applicable to other kinds of skin needs further investigation.

(b) Effects of vitamin A on tissue other than skin in culture.

When the esophagus of the rat was exposed to excess vitamin A in vitro, the differentiation of the esophageal epithelium was drastically modified (Lasnitzki, 1963). Keratinization was inhibited. Instead, the superficial cells were transformed into mucin-secreting elements. After ten days growth all treated cultures showed mucous changes. However, the mucous cells were restricted to the surface layer of the many layered epithelium.

Aydelotte (1963) studied the effect of excess vitamin A on chick embryonic esophagus (2.5-10i.u. vitamin A/ml.). The epithelium acquired a pseudostratified or thin stratified structure, with high columnar ciliated and mucous cells.

When vaginal epithelium of young rats was cultivated in a control medium the cells proliferated and keratinization occured (Kahn, 1954). However, in the presence of vitamin A keratinization was inhibited. Lasnitzki (1961) repeated this experiment on mouse vagina using chemically defined medium (modified Connaught 858) and showed similar results. Complete mucous metaplasia was not observed.

Lawrence, Bern and Steadman (1960) and Lawrence and Bern (1960) implanted for various periods of time paraffin pellets which contained differing concentrations of vitamin A (10^2 to 10^5 i.u. vitamin A/ml. medium) into Syrian hamster cheek pouch. When only a small amount of vitamin A was implanted into the cheek pouch the mucosa showed hypertrophy of the epithelium. The stratum granulosum was thickened and an increase in size of individual granules was seen. When a large amount of vitamin A was applied no keratinization was observed. High columnar

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cells with mucous granules appeared. These metaplastic changes were confirmed by Cavalaris and Krikos (1967) and Cavalaris, Matukas and Krikos (1969) using similar paraffin rods containing 5×10^4 i.u. vitamin A/rod. The metaplastic changes produced by vitamin A appeared to be correlated with the duration of treatment. They observed profound mucous metaplasia after 20 to 25 days. At some regions a single layer of mucous cells overlay one or two layers of basal cells. The paper by Cavalaris et al.,(1969), described the histochemistry of the mucus induced by excess vitamin A. The results indicated that this mucin had staining characteristics which were compatible with the known chemical constituents of normal epithelial mucin.

(c) Hormones and mucous metaplasia.

The occurrence of mucous metaplasia of the vaginal epithelium during pregnancy and pseudopregnancy was first reported in the dog, rabbit, guinea-pig and mouse by Retterer (1892) and Lataste (1892) and in the rat by Long and Evans (1922). Courrier (1924) was the first to induce this change experimentally by the injection of follicular fluid into the immature or castrated adult guinea-pig. Similar results were obtained by Hisaw, Meyer, and Weichert (1928) in castrated rats, and by Weisner and Patel (1929) in castrated mice, by the administration of corpus luteum extracts. Selle (1922) reported that mucous metaplasia occurred in guinea-pigs shortly before the onset of each estrus cycle. However, the hormone(s) involved in this change was not known. Mucous metaplasia can be produced by subcornifying doses of estrin (crude estrogen extract) (Robson and Wiesner, 1933) or estrogen (Meyer and Allen, 1933). If the injection was above the optimum amount cornification of vaginal epithelium resulted, so that mucous cells surmounted the stratified epithelium. MacDonald and Robson (1939) reported similar

findings using synthetic estrogen. The mucous change was increased by giving either progesterone or testosterone along with the estrogen.

It is now well established that the principal effect of estrogen on vaginal epithelium is to stimulate proliferation and produce cornification (Husbands and Walker, 1963; Barker and Walker, 1966), while the effect of progesterone is to produce mucous metaplasia. This is the case during pregnancy and pseudopregnancy in which progesterone activity surpasses that of estrogen. Progesterone causes the vaginal epithelial cells to be transformed from squamous to cuboidal or columnar (Selye, 1940; Barker and Walker, 1966). Recently Eddy and Walker (1969) have studied the hormone controlled differentiation in castrated mice by electron microscopy. They reported that the mucous cell produced by progesterone injection is a typical goblet cell.

(d) [redacted] metaplasia in other organs

Glandular metaplasia is rare in the mucous membrane of the oral cavity. However, a few reports have appeared. Spouge (1966) reported a case of sebaceous metaplasia in the oral cavity associated with dentigerous cyst epithelium. As sebaceous gland is not normally present in the oral mucous membrane its presence in this case must be due to a metaplasia. Krikos (1966) observed mucous metaplasia of an odontogenic cyst which is characteristically stratified squamous type.

In other parts of the alimentary canal glandular metaplasia has more often been observed (Järvi, 1962), and has also been observed in the gall bladder (Järvi, 1962; Järvi and Meurman, 1964). The most common site is the gastric mucosa, in which patches of intestinal mucosa (including cells with brush border and goblet cells) can be seen (Järvi, 1962; Ming, Goldman and Freiman, 1967; Goldman and Ming, 1968). The intestinal metaplasia of the gastric mucosa develops in connection with

chronic gastritis, and seems to play a role in the development of gastric cancer (Järvi and Laurén, 1951). Ming et al., (1967) and Goldman and Ming (1968), who studied the fine structure of the intestinal metaplasia of human gastric mucosa by electron microscopy, also reported the association of metaplasia with gastric adenocarcinoma. They reported that in addition to the fully developed intestinal metaplasia (with goblet cells and columnar absorptive cells), cellular variants suggesting partial or incomplete forms of metaplasia were also observed both in benign and malignant lesions. Feit et al., (1967) were able to induce metaplasia of gastric mucosa experimentally. They reported the transition of the intestinal metaplasia into adenocarcinoma. They regarded this metaplastic change as precancerous.

Intestinal metaplasia has also been reported in esophagus and nose (Järvi, 1944, 1945) and in gall bladder (Kerr and Landrum, 1936; Järvi and Meurman, 1964; Järvi and Laurén, 1967). In gall bladder the intestinal metaplasia was complete (or true) metaplasia. Besides the columnar absorptive and goblet cells, Paneth and argentaffin cells were also observed (Järvi and Laurén, 1967; Kerr and Landrum, 1936). In addition to the intestinal epithelial metaplasia there were mucous secreting glands in the mucosa and in one case metaplasia resembling gastric superficial epithelium and antral glands (Järvi and Laurén, 1967).

Gordon (1963) more recently reported similar (true) intestinal metaplasia in the urinary bladder mucosa.

In addition, pancreatic metaplasia in gall bladder or extrahepatic biliary ducts (Elfving and Hästbacka, 1965; Järvi, 1962; Järvi and Meurman, 1964) and liver metaplasia in gall bladder (Halpert, 1927; Thorsness, 1941) have also been reported.

The epithelium of the uterus was observed by Novak (1932) to

show metaplastic change to the pattern of epithelium normally found in fallopian tubes. In 1946 Holand reported the observation of tubal metaplasia in curetted non-secretory endometrium with hyperplasia. Tubal metaplasia was found in 24 out of 100 routine curetting examinations reported by Fruin and Tighe (1967) covering a wide range of ages. Metaplasia was not found in endometrium during secretory or menstrual phases, nor was it found in post-menopausal atrophic endometrium. When carcinoma was present tubal metaplasia was found in the non-neoplastic area surrounding the tumour.

(e) Mucous metaplasia in urinary tract.

Mucous metaplasia probably occurs more frequently in the urinary bladder than in any other part of the body. It has been observed in association with a variety of conditions including exstrophy (Craiz, 1939; Mostofi, 1954; Scholl, 1922), calculi (Foot, 1944; Mostofi, 1954; Stirling and Ash, 1941; Patch, 1948), and in patient's with urethral strictures (Saphir and Kurland, 1939). Mucous metaplasia involves a change of transitional epithelium to mucous secreting type, very often with mucous glands. The glands are formed of simple columnar cells with basal nuclei and clear cytoplasm, similar to goblet cells of the colon.

The occurrence of glandular metaplasia in the renal pelvis is said to be much rarer and only a few cases have so far been reported (Krag and Alcott, 1957; Ackerman, 1946).

There has been some discussion on the relation of glandular metaplasia and adenocarcinoma in the urinary tract (Craiz, 1939; Mostofi, 1954; Scholl, 1922; Patch, 1948; Krag and Alcott, 1957). In a study of exstrophic bladders Scholl (1922) traced the development of extensive glandular metaplasia through early malignant change to invasive and metastasizing adenocarcinomas. Mostofi, Thompson and Dean

(1955), after studying 44 cases of adenocarcinoma of the bladder, were of the opinion that the tumours originated from sites of glandular metaplasia.

3. Cartilaginous and Bony Metaplasia.

Cartilaginous and bony metaplasia is a common type of metaplasia. It has been observed in almost all the tissues of the body. Because it is so common it stimulated an extensive study of the factors underlying the development of cartilage and bony metaplasia in the nineteen twenties and thirties. However, no conclusive results were obtained and the mechanism for heterotopic chondrogenesis and osteogenesis remains unknown. The following review is to give a general account of cartilaginous and bony metaplasia and some aspects of experimentally produced chondrogenesis and osteogenesis in heterotopic sites.

It is familiar to clinicians that bone may sometimes form in scars, either surgical or due to other causes. Strassburg (1911), Nicholson (1922) and Greig (1933) have reported cases of bony metaplasia after laparotomy. In Strassburg's (1911) case bone was observed 2 1/2 months, while in Nicholson's (1922a) case, only 41 days after operation. In the latter it was firmly calcified and was seen to consist of a branched trabeculae of bone lamellae with Haversian canals. Intermingled with the bone was some hyaline cartilage. Bony metaplasia has been observed much more often in upper than lower abdominal scars (Willis, 1958).

Heterotopic bone developing in the dermis of skin was observed by Lloyd-Williams (1929) in an old burn scar. Lilga and Burns (1942) reported a similar case in a traumatic scar. Nicholson (1922a) observed some bony spicules associated with the hair follicles of the lip. Multiple bony nodules have been reported in the skin of a patient suffering from

severe long-standing acne vulgaris (Leider, 1950). Cartilaginous metaplasia has been shown by Vero, Machacek and Bartlett (1954) in scrotal skin and various other areas of the skin.

Metaplastic cartilage and bone have also been reported in certain other tissues, such as lymph glands, tonsils, thyroid, alimentary canal (Ackerman, 1968) and urinary tract (reviewed below). But it has been said that the most common site of bony metaplasia is the sclerotic artery (Bunting, 1906); it occurs less frequently in the lung and in the dura mater (Halstead and Christopher, 1923).

Bunting (1906) described the aorta from a man of 72 as a rigid tube containing masses of bone underlying atheromatous areas adjacent to the calcified media. The bone contained marrow and haemopoietic tissue. Nicholson (1922b) and Schulze (1929) reported the observation of metaplastic bone in the calcified walls of aneurysms. In addition, cartilage formation in sclerotic arteries has also been documented (Monckeberg, 1902 and Marbury, 1902, from Willis, 1958). Metaplastic bone has also been reported in other regions of the cardio-vascular system. Nicholson (1922b) has observed cartilage and bone in fibrotic or calcified heart valves of patients with chronic or recurrent endocarditis. Farris, Yeakel and Seitner, (1946) have observed in 32 rats metaplastic cartilage attached to the left ventricular wall by a fibrous pedicle.

Metaplastic bone has been observed in skeletal muscle, either after traumatic injury (Constance, 1954), or in a generalized progressive form. The latter type has been reviewed and documented by Fairbank (1950) and Maudsley (1952). This invoives the crippling ossification of muscles and/or tendons, intramuscular septae, and muscle attachments to bones.

In man ossification of tendon is said to follow mechanical injury (Jones, 1932; Hirsch and Morgan, 1939; Mallinson, 1932). Metaplasia has been observed to develop under normal conditions in dense fibrous tissues of various sites, particularly over the attachments of tendons and ligaments to bone (Scapinelli and Little, 1970). The gradual transformation of these tissues to fibrocartilage (Knee and Biermann, 1958; Scapinelli and Little, 1970), or cartilage to fibrocartilage (Amprino and Buirati, 1934), or tendon to bone, as in turkey tendon (Johnson, 1960), has been documented.

Buck (1953) reported the observation of cartilaginous and bony metaplasia in the regnerating Achilles tendon of rats. He also reviewed the few reports made of the development of bone in human tendon after division. The appearance of bone in regenerating tendon suggests that its development is related to the function of transmitting tension. Metaplastic bone was seen to a much lesser extent when the leg of the rat was immobilized after tenotomy (Buck, 1953). Several investigators have pointed out the importance of mechanical forces in ossification (Thoma, 1913; Carey, Zeit and McGrath, 1927-28; Krompecher, 1937; Scapinelli and Little, 1970). Krompecher, after an extensive study, concluded that pressure forces promote the development of cartilage and chondral bone, while tensional forces stimulate dermal bone formation. Scapinelli and Little (1970) believed that combined compression and rotation caused metaplastic changes, stimulating elongated fibrous tissue cells to form round chondrocytes.

(a) Cartilaginous and bony metaplasia related to urinary bladder mucosa.

A large number of reports are concerned with the experimental studies of bony and cartilaginous metaplasia related to the transitional epithelium lining the renal pelvis, ureter and bladder.

Blessing was the first to observe bony metaplasia in renal pelvis in 1859. He reported that in rabbits ligation of the renal pedicle was followed by the appearance of plaques of bone under the epithelium of the pelvic of the kidney. Sacerdotti and Frattin (1902) confirmed that ligation of the renal vessels in rabbits produces massive necrosis of kidney, that was followed by ossification. Liek (1906, 1908) reported that osteogenesis was induced in the kidney within 16-20 days after ligation of the renal artery. These observations have since been substantiated by Pearce (1909), Asami and Dock (1920), Ceccarelli (1931) and Willis (1958). Metaplastic bone was invariably found under the transitional epithelium of the calyces.

Huggins (1931a) was able to produce metaplastic bone beneath pelvic mucosa by fulguration of the renal pelvis with a high frequency electrical current or with 95 percent phenol.

In 1914 Strauss reported that when defects of the ureters of dogs were repaired with flaps of muscle and fascia a layer of bone was found bordering the lumen of the ureter.

Neuhof (1917), during an extensive study on the feasibility of fascia transplantation in repairing vesical defects, found that when fascia was used for repairing bladder bone invariably developed and was confined to the surface of the transplanted fascia that bordered the lumen. Phemister (1923) confirmed Neuhof's observation in the dog but he was not able to reproduce these experiments in the rabbit and sheep.

Huggins and his associates (Huggins, 1930a,b ; Huggins, 1931a, b; Huggins and Compere, 1930; Huggins and Sommet, 1933; Huggins, McCarroll and Blocksom, 1936) conducted a series of experiments concerned with autotransplantation of vesical mucosa of dogs, including renai pelvis, ureter and bladder. He found that when these mucosae were grafted to the

rectus fascia, cysts of transitional epithelium were found beneath mucosa, and bone was found in cyst walls on the side nearest the fascia. Bone was observed only 19 days after transplantation. It was noted that metaplastic bone appeared only beneath the newly proliferated epithelium and never next to the original graft. When scrapings of bladder mucosa were used (Huggins, 1931a) islands of bone were consistently observed. No metaplastic bone was observed when the bladder mucosa was grafted to liver, spleen, kidney or lung, but developed following grafting to adipose tissues, muscle and synovial membrane. These observations have been extended and confirmed by Johnson and McMinn (1956a), Makin (1962), Anspach (1964) and Mestal and Spain (1967).

In 12 patients undergoing two-stage prostatic operations Welcker (1950) implanted pieces of bladder mucosa in the subcutis, fascia or muscle of the abdominal wall. When re-operated 22 days later, bone was found in three cases including one having cartilage nodules.

Homografts of bladder mucosa have shown variable results (Johnson and McMinn, 1956b; Marshall and Spellman, 1957; Mestel and Spain, 1967). Bone developed quickly after homo-transplanting urinary bladder mucosa, but the bone was resorbed when the transplanted mucosa was rejected. Mestel and Spain (1967) therefore concluded that the maintenance of the metaplastic bone requires the continuous presence of a stimulus, presumably from the transplanted mucosa. Similar experiments have been reported for rats (Friedenstein and Lalykina, 1963) which had a much lower yield (10 percent of the animals) of bony metaplasia than guinea-pigs or dogs.

Although osteogenesis has been successfully induced by transplantation of gall bladder mucosa (Huggins and Sammet, 1933), mucosa of other regions failed to induce heterotopic bone formation (Huggins, 1931a).

(b) Experimental induction of osteogenesis by other means.

Ossification in the soft tissues can be produced by methods other than urinary mucosal grafts. It has been successfully obtained by introducing devitalized tissue into the non-skeletal tissue (Urist and MacLean, 1952; Peer, 1955; Bridges and Pritchard, 1958), or by tissue extracts (Levander, 1938; Bertelsen, 1944; Lacroix, 1947, 1951), or by irritants (Hartley and Tanz, 1951). The results obtained by the injection of tissue extracts were not consistent as bone was not invariably induced. Heinen, Dabbs and Mason (1949) were quite critical about these results. They were able to induce cartilaginous and bony metaplasia by the single injection of alcohol alone. They found that after an intramuscular injection of 40 percent alcohol, 23 out of 37 of injected triceps (62 percent) and 12 out of 40 injected quadriceps (30 percent) contained cartilage or bone. Huggins et al., (1936) had earlier been unable to induce osteogenesis by the injection of extracts of urinary bladder mucosa.

When cultured human amniotic cells of the FL line were injected into the thigh muscle of the cortisone-conditioned ICR/Ha mice, bone formation was induced within 12 days (Anderson, Merker and Foch, 1964; Anderson and Coulter, 1965, 1967; Anderson, 1967). Cartilage or hyaline type was observed as early as six or seven days after the FL cell injection. This finding has recently been confirmed by Wlodarski, Hinek and Ostrowski (1970), in various strains of mice other than the ICR/Ha used by Anderson and his associates. In addition, they reported that another line of cultured human amniotic cells, known as WISH, had the property of inducing chondrogenesis and osteogenesis.

Cartilage and bone formation evoked by FL or WISH cells is in some aspects similar to bone induced by grafts of urinary bladder mucosa.

(Huggins, 1931a). The main difference is that bladder mucosa primarily evoked bony tissue and only occasionally cartilage, whereas FL and WISH cells induced chiefly cartilage, and bone only appeared later.

(c) Factors underlying cartilaginous and bony metaplasia.

Although a lot of effort has been made in this direction the reasons for the change are still not known. Huggins (1931b) noted that alkaline phosphatase activity was high in transplanted bladder epithelium at the time of bone formation, and was slight in the non-proliferated original graft.

Constance (1954) used a concentrated aqueous extract of foetal guinea pig bones having very high alkaline phosphatase activity but failed to induce metaplastic bone or cartilage in the muscle.

Using histochemical methods Friedenstein (1959) found a PAS positive substance in areas of early new bone formation, accompanied by the disappearance from the epithelial cysts of urinary bladder transplants of a substance with the same staining characteristics. Makin (1962) confirmed these findings in another study. In addition, he demonstrated that the fluid content in the cysts contained an "osteogenic substance". When the cysts were punctured and the fluid allowed to ooze to the nearby tissue, a bigger area of metaplastic bone developed. This was substantiated by Alverez-Ierena (1952). He found also that in the normal dog a mucoprotein which is not found in the plasma was added to the urine, presumably from the transitional epithelium. Whether or not this mucoprotein is responsible for metaplastic change requires further investigation.

Anspach (1964) devised experiments in an attempt to find out, by isolating the grafted mucosa from the fascia with millipore filters, whether the induction of osteogenesis by bladder epithelium was due to

direct epithelial contact or to some diffusible substances. The negative results of his study led him to conclude that it is not likely that a diffusible substance with osteogenic capacities exists in vesical mucosa and that the actual physical proximity of the two tissues is a factor in this form of bone induction.

On the other hand, Friedenstein and his associates (Friedenstein, 1962; Friedenstein et al., 1967) were able to induce osteogenesis by isolating the transitional epithelium with millipore chamber. In addition, they also succeeded in inducing bone formation by transitional epithelium within the millipore chamber, of cells from peritoneal fluid or spleen (Friedenstein et al., 1967; Friedenstein, 1968). These results would seem to indicate that there is a diffusible substance produced by transitional epithelium, which is responsible for heterotopic bone induction; and the bone cells might be of haemopoietic origin.

(d) Bony metaplasia of cartilage tissue.

This review has so far described how, under certain circumstances, non-skeletal tissues can undergo metaplasia and form cartilage or bone. But whether or not cartilage can redifferentiate and become bone has attracted little attention. It was assumed that cartilage must be completely resorbed and replaced by calcified bone (Ham, 1930 and Roussekoff, 1959). On the other hand, some authors believe that redifferentiation of chondrocytes to osteocytes occurs under certain circumstances (Bohatirchuk, 1965, 1969; Bloom and Fawcett, 1968; Wheeler and Mohuiddin, 1968). Wheeler and Mohuiddin (1968), after studying maturation of the epiphyseal cartilage plate of long bones, concluded that the epiphyseal cartilage plate is converted to bone and not replaced by it.

Bohatirchuk (1969) used "stain historadiography" (combined

histology and x-ray investigation) to study the callus formation after experimental factures in rats and rabbits. He reported the observation of the development of metaplastic bone from existing chondrocytes of the callus. The difference between the trabecular bone and metaplastic bone was that the latter did not have canaliculi (Bahatirchuk, 1969).

It is difficult to come to a conclusion on this point because of the relatively limited resolution of the light microscope and the possibility of confusion of calcified cartilage with metaplastic bone.

Aside from the literature reviewed above, there are many reports concerning cartilaginous and bony metaplasia in tumours of various tissues and organs (see Willis, 1958). Metaplasia was found around the necrotic stroma of the tumour, or as metaplasia of sarcoma in situ. No attempt will be made to review these reports here.

III. MATERIALS AND METHODS

1. Vitamin A Deficiency

Young wistar rats (Woodlyn Farms, Guelph, Ontario) weighing 50 to 100 gm. at the start of the experiment were caged individually and fed a vitamin A deficient diet (Nutritional Biochemicals, Cleveland, Ohio). Tap water was available ad libitum. A total of 80 vitamin A deficient rats (both male and female) and four control animals kept under the same conditions, were used but maintained with complete stock diet (Purina Lab. Chow). The temperature of the room was maintained at 75° F.

Animals were sacrificed from the end of the first to the twenty second week on the diet by injecting a lethal dose of sodium pentobarbital.* The trachea and the urinary bladder were quickly removed, opened longitudinally and fixed for four to six hr. in chilled 2.5 or 5 percent glutaraldehyde solution, buffered with 0.1M Sorensen's phosphate buffer at pH 7.3. After fixation, the tissues were washed in several changes of buffered sucrose (5.4 percent) solution.

For studying the trachea, the bottom 1/3 of it was cut into pieces about one by two mm. They were then osmicated with one percent OsO_4 for two hr. and processed for electron microscopy, as described below.

The bladder was examined grossly or under the dissecting microscope for areas of leukoplakia. These areas were removed and cut into small pieces. Small pieces of tissue from varying regions of bladder which did not show any gross changes were similarly treated.

*Nembutal, Abbott Laboratory

The tissues were dehydrated in acetone and embedded in Vestopal W (Jaeger, Geneva) or in Durcupan ACM9 (Fluka, A.G.). The pieces were oriented so that the trachea or the bladder could be cut transversely and areas of interest were thus localized by examination of thick sections with the light microscope. Thin sections were cut on a Sorval Ultra-microtome MT 1 or 11 and collected on 75 mesh grids coated with formvar and carbon. Sections were stained with lead citrate (Reynolds, 1963), or saturated aqueous uranyl acetate (Watson, 1958), or with both and examined with an RCA EMU 3D electron microscope operated at 65 KV or with an AEI 801 at 60 KV.

2. Staining with Ruthenium Red.

The procedure of Luft (1965b) was used with few modifications. Small pieces of normal urinary bladder tissue were fixed for four hours in a solution containing one part of eight percent glutaraldehyde, one part of 0.3 percent ruthenium red (purified, Luft, 1965b). Tissues were rinsed with three changes of 0.1 M cacodylate buffer over a period of 15 min. The tissues were then post-fixed for two hr. with a solution containing one part of five percent OsO_4, one part of 0.2 M cacodylate buffer (pH 7.3) and one part of 0.3 percent ruthenium red. The tissues were rinsed briefly in buffer solution and dehydrated as usual through graded ethanol and embedded in Durcupan.

3. Chick Embryo Culture.

(a) Material

Fertilized chicken eggs were incubated at 37 ± 1°C. for six, seven, eight or nine days. The embryos were removed aseptically and rinsed in two changes of Hank's balanced salt solution (B.S.S.). Skin

of the back of the embryo was removed with iris scissors and divided into pieces two to three mm. in diameter. They were rinsed once again in B.S.S. before being transferred to the culture medium.

(b) Culture methods.

The raft technique for organ culture was employed (Moscona, Trowell and Willmer, 1965). Falcon plastic organ culture dishes (60 x 15 mm.) (Falcon Plastics, BioQuest, California) with a stainless steel supporting grid in the central well were used. In order to avoid the growth of skin tissue into the mesh of the grid, a sheet of sterile siliconized Ross optical lens paper (A. Rosmarin, Brooklyn, N.Y.) was laid over the steel grid. Culture medium was added gently to the centre well so that the raft was in contact with medium but not submerged. The absorbent disc in the outer well was moistened with approximately five ml. of sterile distilled water.

The tissues were then pipetted on to the lens paper with a minimal amount of culture medium. Excess medium around the tissues was removed by a fine drawn pipette. Care was taken to assure that the skin was oriented with epidermis uppermost. Usually four explants were cultured on a single raft. They were kept in a humidified incubator at 37° C, with a gas phase of 95 percent air and five percent CO_2. The culture medium was replenished every two or three days.

(c) Media

The media used for these investigations were McCoys 5a and Waymouth Medium MB 752/1. The former, in addition, contained 30 percent calf serum, while the latter was a chemically defined medium (see appendix).

(d) Additions of vitamin A to the medium.

Pure crystalline vitamin A alcohol (Retinol, Nutritional Biochemical Corporation) was dissolved in ethanol and added to the

culture medium less than 24 hr. prior to each experiment. The concentration of vitamin A in the final medium was adjusted to contain 7.5, 10, 15, 20, 30 and 40 i.u. vitamin A per ml. The final concentration of ethanol was kept at or below 0.2 percent. The control cultures contained exactly the same medium with the exception that no vitamin A was added to the media. The cultures were fixed and examined for epithelial metaplasia after various periods of incubation.

4. Hamster Cheek Pouch

Thirty young adult (150 gm.) male golden Syrian hamsters were used.

Fisher's low melting point paraffin (M.P. 48° C.) was used. The melting point of this paraffin was further lowered (to 42° C.) by adding one part of mineral oil to seven parts of paraffin. Paraffin pellets were made in such a way that each contained 15 mg. (50,000 i.u.) of vitamin A.

To make the paraffin-vitamin A rods, 150 mg. of crystalline vitamin A alcohol (retinol) was dissolved in two ml. of diethyl ether and this was then added to 10 ml. of the molten paraffin-mineral oil mixture (at about 42° C.), mixed thoroughly, and cast into individual glass tubes of size 2.0 x 0.8 cm. These were kept in the freezer (-15° C.) until used. All paraffin pellets were used within eight hr. of being made.

The animals were anaesthetized by an intra-peritoneal injection of sodium pentobarbital (0.06 mg./gm. body weight). The area of the left pouch from the labial commissure to the shoulder girdle was shaved with an electric clipper. The pouch was everted and cleaned with cotton swabs and distilled water. The pouch was then replaced, together with a cotton

swab, into its original position. The pouch contained a cotton swab so that it could be identified at a later stage of the procedure.

A two cm. vertical incision was made approximately 1.5 cm. posterior to the labial commissure. The pouch was pushed through the external incision with the aid of the cotton swab and the terminal muscle attachment to the pouch was severed. The cotton swab was then removed and replaced with a paraffin-vitamin A pellet through the oral cavity. A loop suture, which was made at the neck of the pouch through the incision, secured the pellet within the pouch. The skin incision was closed with 3-0 silk sutures.

The animals were sacrificed every five days up to 25 days with an excess of sodium pentobarbital. The portion of the pouch distal to the suture was removed. Selected samples from different areas of the pellet-treated pouch were fixed in glutaraldehyde, then OsO_4, and processed for electron microscopy, following the procedures mentioned in the preceding section.

5. Mouse Vaginal Epithelium

Strain A female mice were used in this investigation. They were ovariectomized at least two weeks before the experiment. Vaginal smears were taken daily from the castrated animal. Only those animals which consistently lacked cornified cells were selected.

(a) Preparation of hormonal solutions

(1) Estradiol benzoate was dissolved in sesame oil to make a concentration of 10 µg./ml.

(2) Because of the required final high concentration of the progesterone solution, direct dissolving of progesterone in sesame oil was not possible. It was first dissolved in acetone, before being added to

sesame oil. Fifty mg. of progesterone were dissolved in five ml. acetone. It was then added to 10 ml. of sesame oil. The acetone was evaporated by leaving the solution in a 60° C. oven for one hr.

(3) To prepare a solution containing both estradiol and progesterone, 50 mg. of progesterone were dissolved in five ml. of acetone and to this was added 10 ml. of sesame oil containing one ug./ml. of estradiol benzoate.

The experimental animals were divided into three groups. Daily injection of hormones was made subcutaneously. In the first group each animal received 0.2 ug. of estradiol in 0.02 ml. sesame oil. In the second group one mg. progesterone in 0.2 ml. sesame oil was administered to each animal. In the third group animals were treated with a combination of estradiol and progesterone (0.2 ug. estradiol and one mg. progesterone in 0.2 ml. sesame oil).

The animals were sacrificed by cervical dislocation every day up to seven days after hormone treatment was begun. Samples of vaginal mucosa were removed and processed for electron microscopy.

6. Tendon

Young adult Wistar rats (150-200 gm.) were used. Under ether or sodium nembutal anaesthesia, the skin of the right Achilles tendon of the rat was shaved, the region cleaned with 70 percent alcohol, and an incision of about 0.5 cm. was made at the right lateral malleolus. By blunt dissection the Achilles tendon was isolated. It was lifted with a hook and transected with a sharp sterile razor blade. Since the incision was so small, no sutures for the skin were needed.

For light microscopic study the tendons were removed at one, two, three, four or five weeks after tenotomy and fixed in 10 percent

formalin overnight. They were embedded in paraffin and stained with 0.1 percent toluidine blue to demonstrate the metaplastic sites by metachromasia. A total of 40 animals were used for this part of the experiment.

For the transplantation studies, animals at various stages (one, two, three, four or five weeks) of tendon regeneration were anaesthetized by sodium pentobarbital. The skin over the regenerating tendon was shaved and cleaned, the skin opened, and the whole regenerating tendon, including the old tendon stump, was removed and placed in Hank's solution. The wound was closed with 3-0 silk sutures.

An incision of about 1 1/2 cm. was then made in the shaved and cleaned skin of the back. By blunt dissection, room for the excised tendon was made available in the subcutaneous tissue. The tendon was then inserted, and the wound closed by wound clips. The animals were sacrificed one, two, three or four weeks after transplantation. The grafted tendons were removed and fixed for light microscopy. A total of 116 animals were used for this part of the experiment.

7. Electron Microscopic Study of Cartilaginous and Bony Metaplasia of the Regenerating Tendon

The tendons at various stages of regeneration were removed and immersed immediately into chilled 2.5 or 5 percent glutaraldehyde. The tendons were sliced longitudinally with a sharp razor blade while immersed in the fixative. After fixation for four to six hours the specimens were rinsed with sucrose solution, and pieces taken from various regions were postfixed in OsO_4 and embedded in Epon 812 (Luft, 1961), Vestopal or Durcupan, as previously described. Forty animals were used for this section of the experiment.

IV. RESULTS

(1) Squamous Metaplasia of Tracheal Epithelium

The epithelium of the normal rat trachea is a pseudostratified type in which four kinds of cells are identified in electron micrographs: basal, ciliated, goblet, and brush-bordered cells (Rhodin, 1959; Rhodin and Dalhamn, 1956). Of these, the ciliated cells and goblet cells are considered to be highly differentiated, while the basal cells are generative (Rhodin, 1966). It has been suggested that the brush-bordered cells represent a stage in the differentiation of ciliated cells (Rhodin, 1959), although recent studies of ciliogenesis (Kalnins and Porter, 1969; Sorokin, 1968) make this appear unlikely. Brush-bordered cells containing developing cilia have not been observed, as might be expected if these cells were differentiating ciliated cells.

Although, by definition, metaplasia is a change affecting already differentiated cells (Ham, 1969) it is observed that the basal cells, rather than the differentiated cells, undergo changes which are truly responsible for producing the metaplastic epithelium. However, certain changes do occur in the fully differentiated cells, and these changes will be described before proceeding to describe the sequence of steps to metaplasia originating in the basal cells.

(a) Effect of vitamin A deficiency on fully differentiated epithelial cells.

Minimal deviations in these cells were observed in animals

deficient in vitamin A for as short a period as one week. Later, many of the cells that lined the trachea lacked the highly specific features of the normal cells (Fig. 1). They had neither cilia, nor mucous granules, nor well developed microvilli. Some ciliated cells contained abnormal basal bodies and intracytoplasmic cilia (Fig. 2). Typical goblet cells were rarely seen, but a type of cell containing dense cytoplasm, many ribosomes and remnants of mucous granules were believed to represent a de-differentiated goblet cell (Fig. 3).

(b) Metaplastic transformation of epithelium

The degree of response to vitamin A deficiency was found to vary greatly from one animal to another. Even in a single animal the changes were often localized. For these reasons it is not possible to relate the changes very closely to number of days on the diet. Rather, they are reported as a sequence of steps in which the epithelium becomes progressively more similar to epidermoid epithelium.

(i) Hyperplastic nests of basal cells

Groups of five, six or more cells in a single section were frequently observed between the basement membrane and the surface cells (Fig. 4). Such cell clusters were seldom seen in normal trachea. Except for an apparently higher content of cytoplasmic fibrils, they showed essentially normal cytological features.

(ii) Stratification

Extensive areas of piled-up cells appear to represent a spread of the basal cell proliferation. The entire epithelium now appeared to be composed of two to six layers of identical proliferated basal cells (Fig. 5). All resemblance to respiratory epithelium was lost as the original surface cells were desquamated. The new cells were cuboidal, with numerous microvilli projecting into wide intercellular spaces.

List of Abbreviations

AUM	asymmetric unit membrane
Bb	basal body
BC	basal cell
BM	basement membrane
C	cilia, centriole, ciliated cell
CC	cell coat
Cf	cytofilaments
Cry	crystalloid, crystallites
CM	calcified matrix
COL	collagen
cv	ciliary vesicles
D	desmosome, dermis
DC	distended cisternae
DCB	dense cortical band
DMV	double membraned vesicle
E	epidermis proper
f	keratin fibrils
FV	fusiform vesicles, angular vesicle
FZ	fibrillary zone
G	golgi, goblet cell
GC	granular cell
GL	glycogen
HC	horny cell
I	intermediate layer

IC	intermediate cell
ICP	intercellular plate
ICS	intercellular spaces
KH	keratohyalin granules
L	lumen, lacunae
LIP	lipid, lipoid
LP	lamina propria
MAC	macrophage
MAT	matrix
MC	mucous cell
MCG	membrane-coating granules
MUC	mucus
M+v	matrix vesicles
Mv	microvilli
N	nucleus
NC	non-keratinized cell
Nu	nucleolus
P	periderm
PC	pre-chondroblast
PG	peridermal granule
S	squamous layer
Sbp	subperiderm
SC	stratum corneum
SG	stratum granulosum
SS	stratum spinosum

T I	type I
T II	type II
T 1	type 1
T 2	type 2
TJ	tight junction
TM	thickened membrane
V	vesicle

All pictures except figures 95, 96, 97, 98 and 99 are electron micrographs. Unless otherwise indicated, all E.M. materials were stained with uranyl acetate followed by lead citrate.

Figure 1. Electron micrograph of tracheal epithelium of four-week vitamin A deficient rat, showing morphological deviations of tracheal epithelium. Brush-bordered (B) and basal cells appear normal but ciliated and goblet cells can not be identified. BM, Basement membrane. Vestopal, uranyl acetate (UA) and lead citrate (LC).

X 5450


1


1

Figure 2. Field showing a small portion of a deviated ciliated cell. In addition to the few cilia present on the surface, an intracytoplasmic cilium is also shown. Lysosomes, Golgi apparatus and what appear to be a second intracytoplasmic cilium can also be identified (arrow). 5-week vitamin A deficiency. Vestopal, UA and LC.

X 18,560

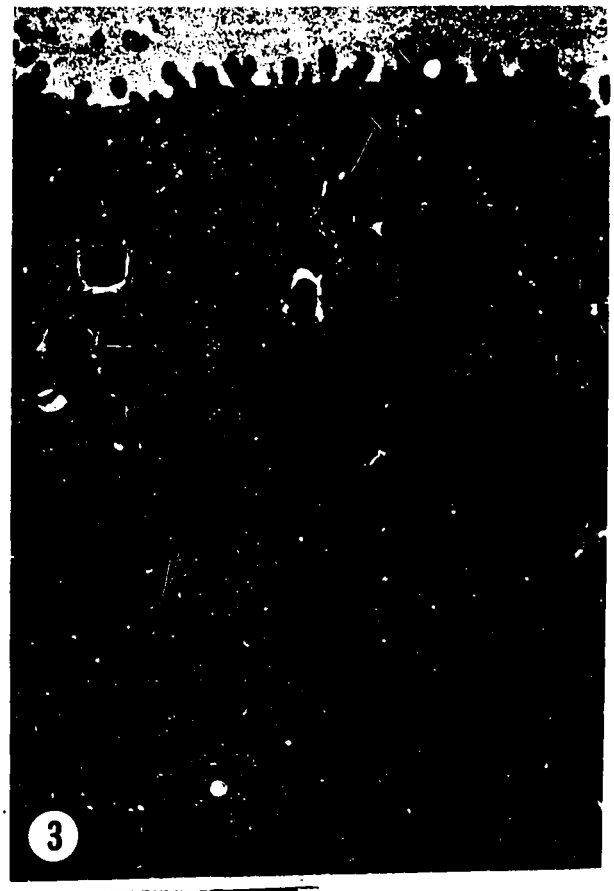
Figure 3. Electron micrograph shcwing apical region of an atypical goblet cell. A few sparsely distributed mucous granules can be seen. 17-week A deficiency. Vestopal, UA.

X 10,490

Figure 4. A group of basal cells piled against each other deep to the respiratory epithelium. BM, Basement membrane. 4-week vitamin A deficiency. Vestopal, UA and LC.

X 6,030


2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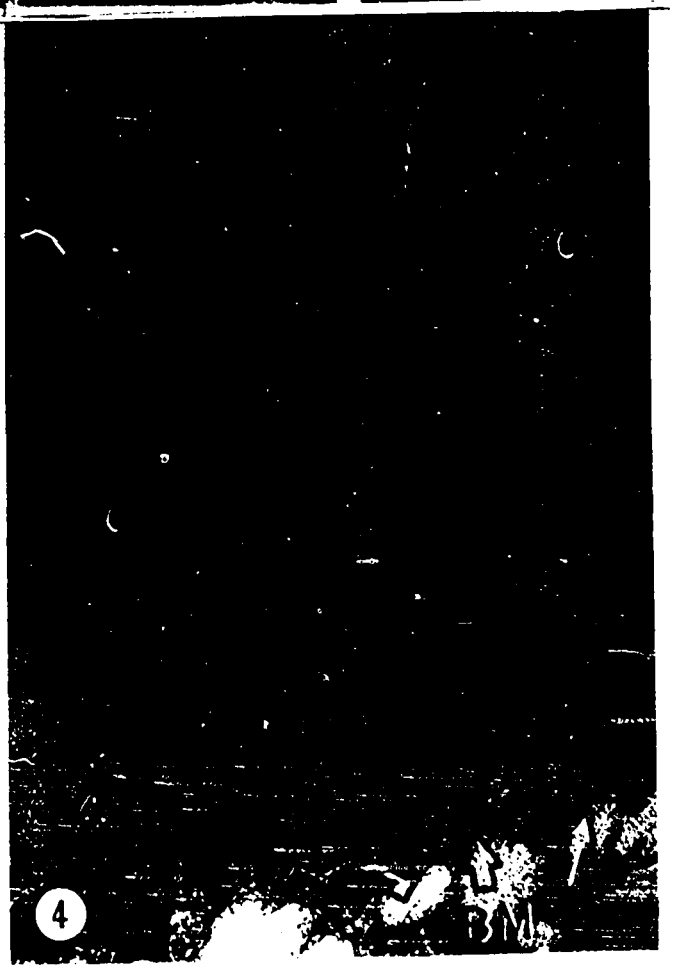

4
BM

(2)

(3)

(1)

Some of these projections terminated as desmosomes from which tightly packed fibrils radiated back into the cells.

The nuclei were large, with prominent nucleoli. Intracytoplasmic membranes were sparse, but many polysomes packed the cytoplasm. These are the features of cells which are rapidly dividing, and mitotic forms were commonly seen.

In images having up to 20 layers of cells there was an obvious difference between the deep and superficial cells. Against the basement membrane were high cells with relatively few fibrils and few microvilli (basal cells). In an intermediate position were several layers of the loosely arranged cells with many microvilli, desmosomes and fibrils (intermediate cells). The most superficial layer was seven or eight flattened cells in thickness (squamous cells) (Fig. 6). A striking feature of the squamous cells was the presence of numerous parallel bundles of filaments (hereafter referred to as "keratin filaments").

Another conspicuous feature of the squamous cells was the many oval vesicles of 80-150 mμ (Fig. 6). The membrane on the inner surface of the vesicles appeared to be coated with finely granular material (Figs. 6,7). Vesicles of slightly different morphology have been described in epidermis (Odland, 1960; Farbman, 1964; Matolsty and Parakkal, 1965; Rowden, 1966) where they were considered to be involved in the production or transport of cell coat material ("membrane coating granules") (Matoltsy, 1966). On the basis of the subsequent development of these vesicles, this term will be applied here. In the metaplastic tracheal epithelium most of the vesicles were located towards the luminal side of the cells (Fig. 6). Such a polarized distribution was particularly obvious in the cells close to the lumen. On the other hand, the Golgi apparatus (GA) was inconspicuous in the most superficial cells,

Figure 5. Result of basal cell hyperplasia. All resemblance to respiratory epithelium is lost. The intercellular spaces are wide. Cytoplasmic projection of adjacent cells intermingled with each other. Some of them terminate on adjacent cells or microvilli of adjacent cells with desmosomes. BM, Basement membrane. 2-week vitamin A deficiency. Vestopal, LC.

X 7900

Figure 6. Part of squamous cell layer. Note the large number of vesicles (arrows) which are located mostly on the luminal side of the cells. The vesicles have an average diameter of 80-150 mμ. A partly desquamated cell (Des) is also noted. 16-week vitamin A deficiency. Durcupan, UA and LC.

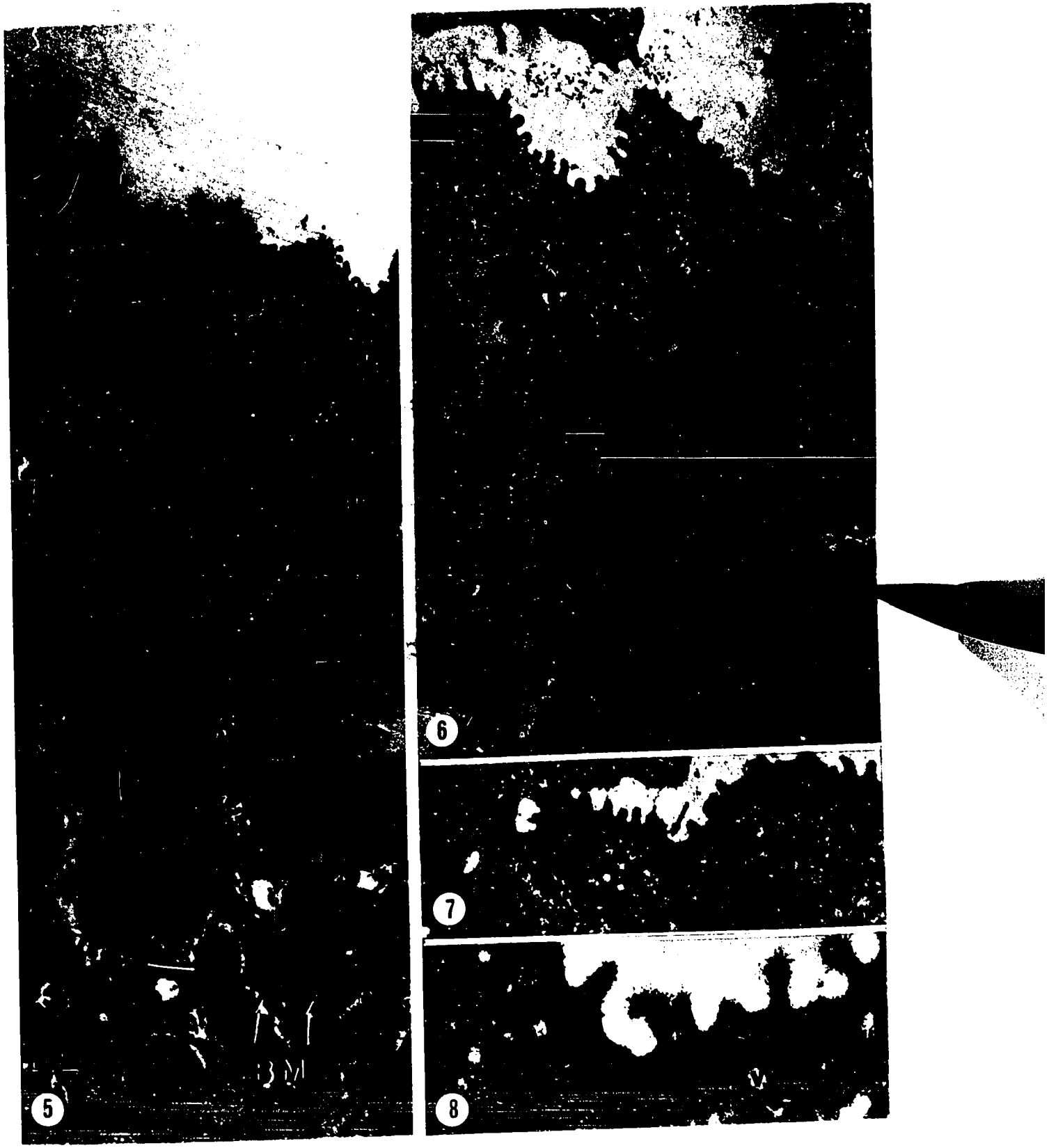
X 23,200

Figure 7. Part of squamous cell. Fusion of a vesicle with the plasma membrane is indicated by an arrow. 16-week vitamin A deficiency. Durcupan, UA and LC.

X 23,000

Figure 8. The plasma membrane and microvilli of a cell in the squamous layer were covered by a surface coat projecting into the lumen of the trachea. In the underlying cytoplasm is a vesicle (V). Note the resemblance of the membrane surrounding the vesicular profile to the plasma membrane. 15-week vitamin A deficiency. Durcupan, LC.

X 43,000


5
6
7
8

BM
5

Dos
6

7

V
8

but prominent in the cells of the deeper part of this layer. It has been suggested that membrane-coating granules take origin from the GA (Bonneville, Weinstock and Wilgram, 1968). The interpretation of these findings is that the vesicles, formed through the activity of the GA in the deeper cells of the squamous layer, then take up a position against the inner plasma membrane. Images interpreted as showing the fusion of the vesicles with the plasma membrane of the luminal surface of the cell were not uncommon (Fig. 7). Moreover, the short, irregular microvilli found on the luminal side of these cells were observed to be coated with finely granular material (fuzzy coat) (Fig. 8), similar to that on the inner surface of the vesicles.

A few very dense large granules, which subsequent events showed to be keratohyalin granules, were also seen in the squamous cells. Their further development is discussed below.

The ultimate stage of stratification before the onset of cornification was characterized by many layers of closely packed epithelial cells (Fig. 9). A progressive increase was seen in the numbers of desmosomes, keratohyalin granules, keratin filaments and membrane coating granules. Desquamating non-cornified cells were observed at the surface, and it is concluded that this stage represents a constantly renewing epithelium.

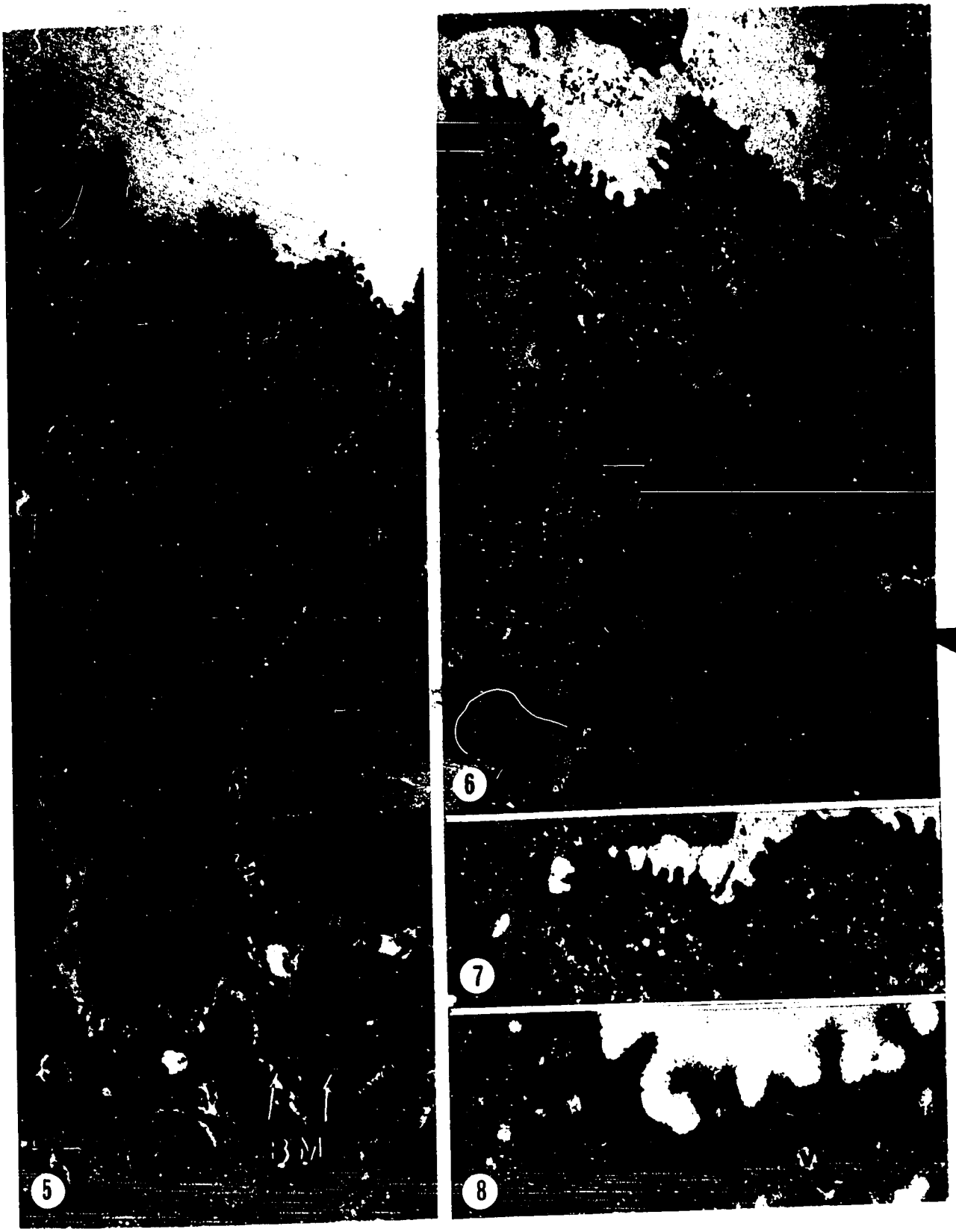
(iii) Cornification

It was possible to recognize, in the fully cornified epithelium, all the layers which are found in epidermis.

(1) Stratum basalis: The principal change from the non-cornified epithelium was an increase in the amount of fibrillary material. Tonofilaments aggregated to form fibrils, except in the immediate perinuclear cytoplasm.

Figure 9. A montage of non-cornified squamous epithelium. The epithelium can be divided into basal (B), intermediate (I) and squamous (S) layers. The basal cells which are separated from the lamina propria of the trachea by a basement membrane (BM) have rather big nuclei, which contain one or more nucleoli. The cells of the intermediate layer are stellate in shape, with prominent nucleoli and keratin fibrils. Membrane-coating granules (arrows) in the inner intermediate cells and also the squamous cells can be identified even at this magnification. Squamous cells are either low, cuboidal or flat. Membrane-coating granules, and also some small keratohyalin (KH) granules are present. The intercellular space is small, and the desmosomes are numerous and well developed. Keratin is quite prominent. However, the horny cell layer has not appeared. 19-week vitamin A deficiency. Vestopal, UA and LC.

X 5300


5
6
7
8


nes
BM
V

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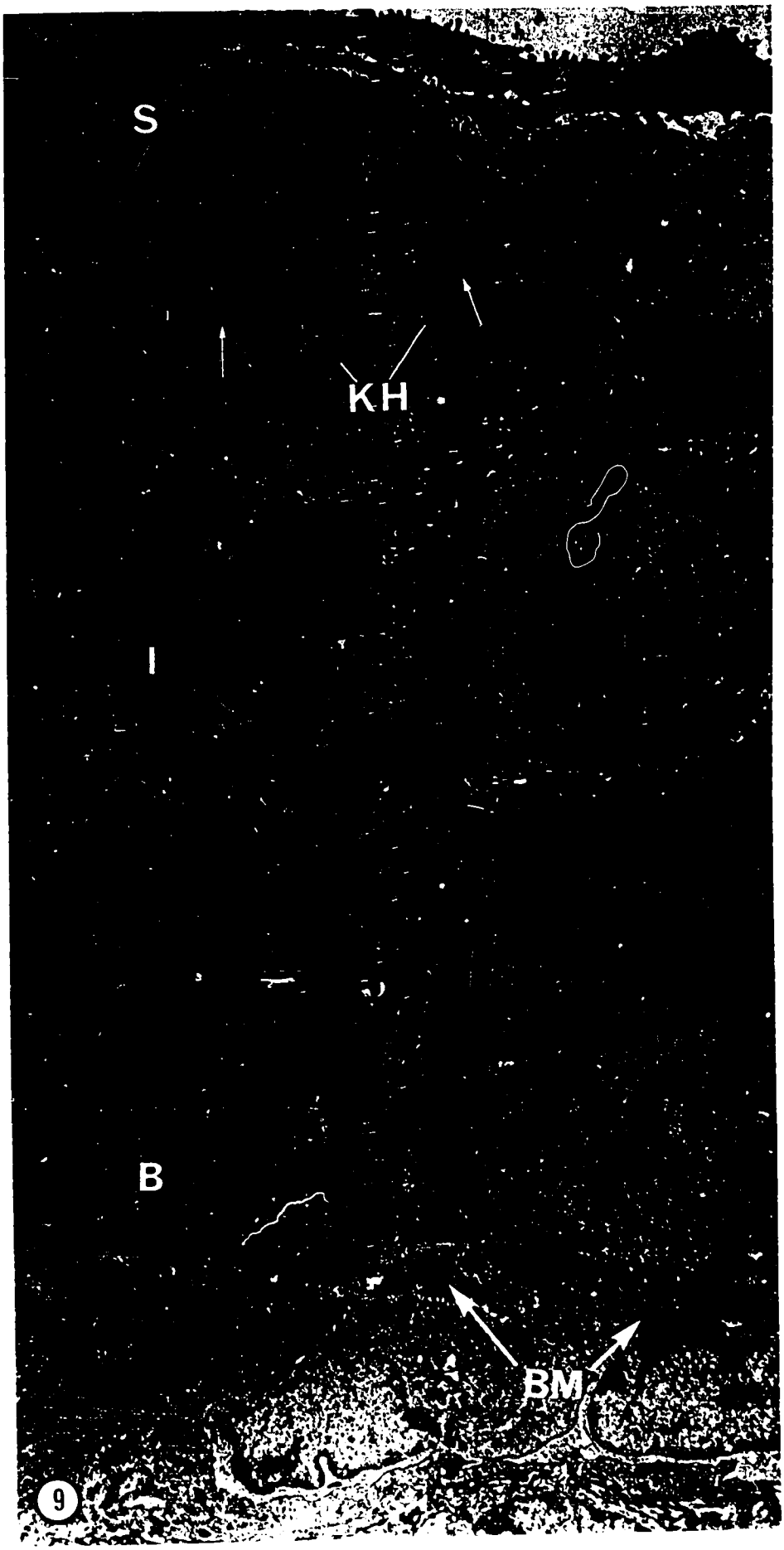
X 5300


S
KH
I
B
BM
9

Also seen in a few sections were other cells interspersed with the basal cells, having the characteristic granules of Langerhans cells, also found in epidermis. The detailed appearance of Langerhans cells in metaplastic epithelium will be reported in a separate section.

(2) Stratum spinosum: The cells differed from those of the intermediate layer of non-cornified epithelium. The fibrillary material was much more plentiful; the membrane-coating granules showed a further increase in density and the development of typical lamellations (Fig. 10). The keratohyalin granules, previously found only in the superficial squamous cells, were now observed in the more superficial cells of this layer. Desmosomes were well developed, and of typical epidermal type. (Fig. 11).

(3) Stratum granulosum: The cells were extremely flat. The conspicuous feature was the large number of keratohyalin granules. These granules, which were dense, unbounded, and of various sizes and shapes, were remarkable in being found not only in the cytoplasm, but also within the nucleus (Fig. 12). Membrane-coating granules were prominent, except in the layer immediately against the stratum corneum (Fig. 12). Discharge of these granules into the intercellular space was inferred from the appearance of their fusion with the plasma membrane of the luminal surface of the cell, and also from the presence of membrane-coating granules in the intercellular spaces.

(4) Stratum Corneum: This layer varied from two to many plates in thickness. The plates were extremely dense, and intact nuclei or usual organelles were not seen (Fig. 13). Occasionally, discrete fibrils and keratohyalin granules and remnants of nuclei were observed (Fig. 13). The plasma membrane was thicker than that of non-cornified cells, measuring about 110A°. The desmosomes of the cornified layer differed

Figure 10. Membrane-coating granules in spinous cells close to stratum granulosum. They are oval in shape. Lamellae occur in granules indicated by arrows. 19-week vitamin A deficiency. Vestopal, UA and LC.

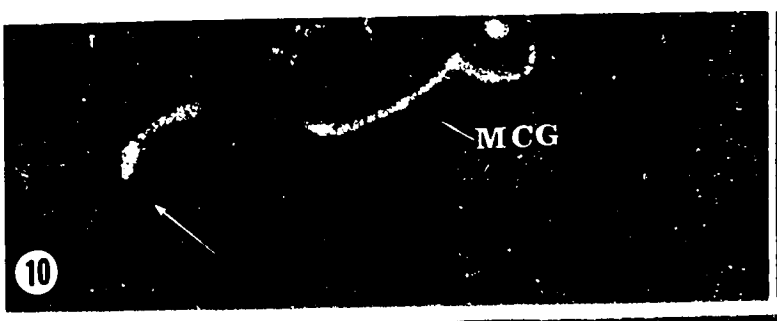
X 34,200

Figure 11. Desmosomes of cells of stratum spinosum. This is the typical desmosome found in the cornifying stage. 22-week vitamin A deficiency. Vestopal, UA and LC.

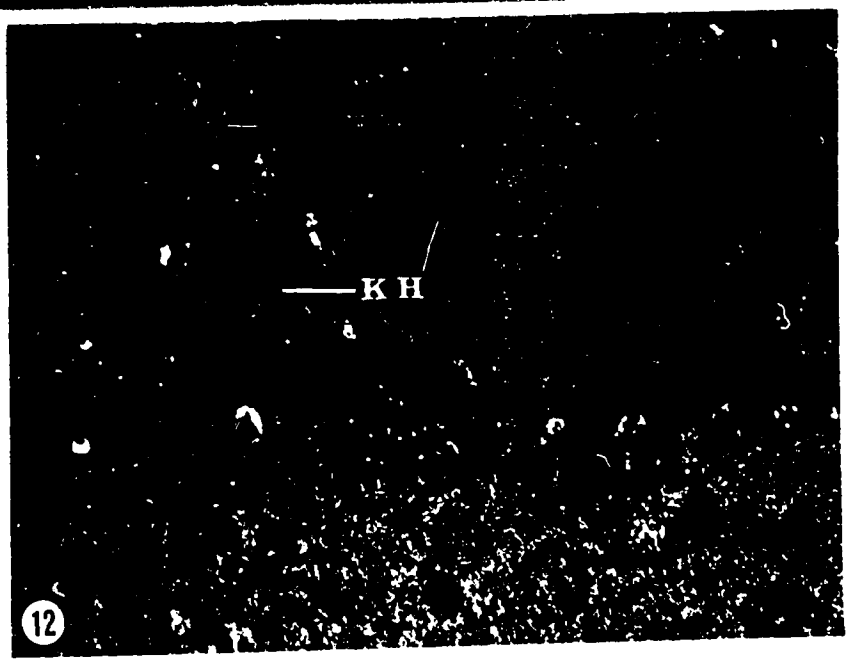
X 43,100

Figure 12. Cells of stratum granulosum. A large number of small very dense granules are seen both in the cytoplasm and in the pynotic nucleus. They were not membrane-bounded. The large granule is also a keratohyalin granule (and not an artefact). 22-week vitamin A deficiency. Vestopal, UA and LC.

X 18,000


MCG
10


11


KH
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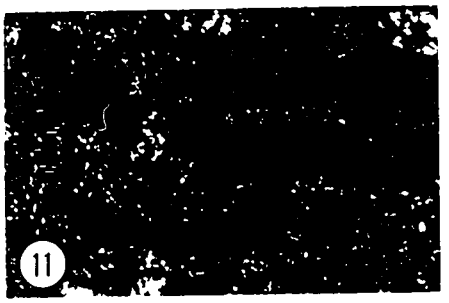
10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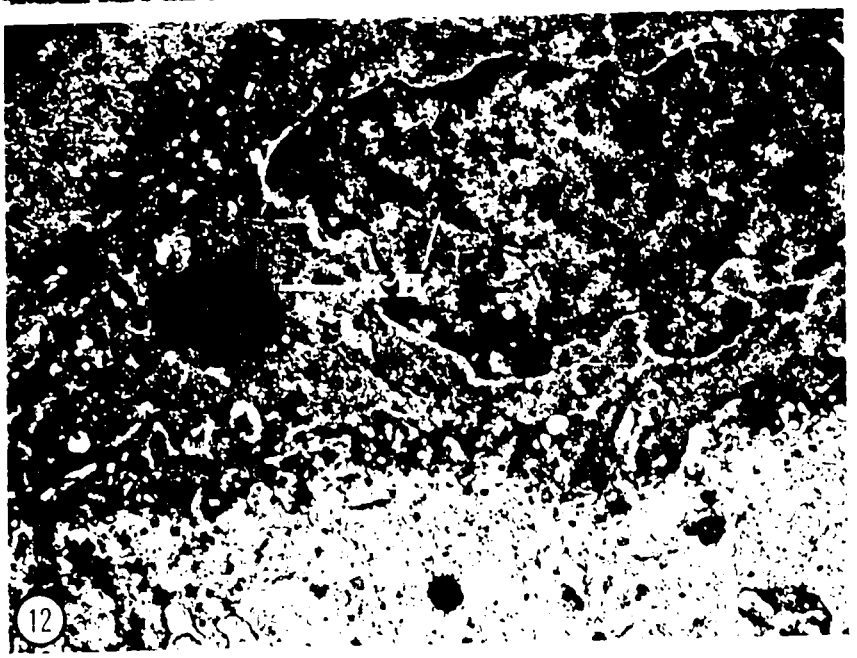
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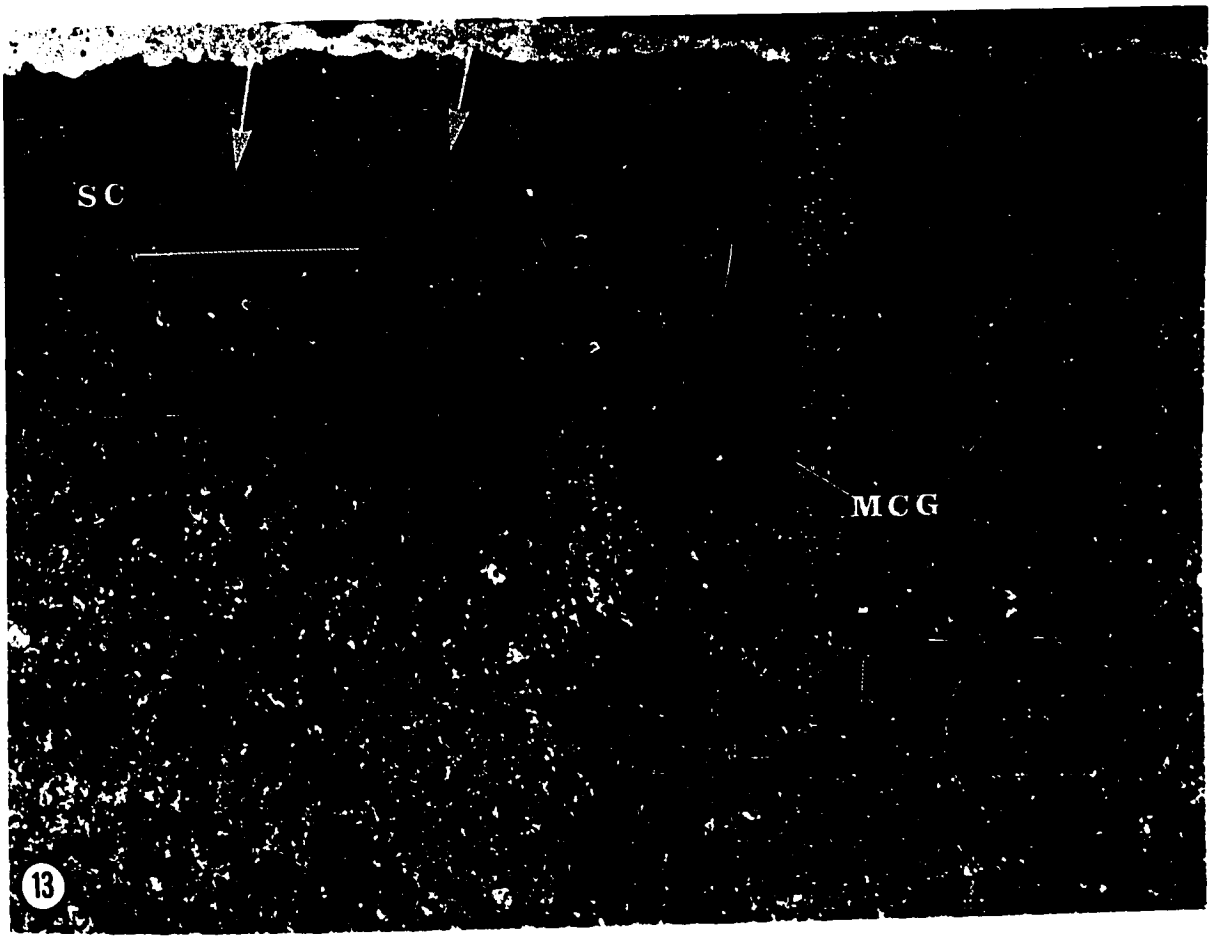
Figure 13. Stratum corneum and stratum granulosum of the cornified epithelium. Remnants of keratohyalin granules (arrows) are present in the horny cells and cells of stratum granulosum. Membrane-coating granules (MCG) are prominent in the cells of stratum granulosum except in the layer just deep to the horny cell. 22-week vitamin A deficiency. Vestopal, UA and LC.

X 13,800


SC
MCG
13


SC
MCG
13

from conventional desmosomes in that the attachment plates became embedded in this thick plasma membrane, but the intercellular disc of the desmosome (the material that lies between the attachment plates) was not only present between the attachment plates but also extended beyond them into the intercellular spaces.

(c) Junction between metaplastic and non-metaplastic epithelium.

The boundary between non-metaplastic and metaplastic epithelium was abrupt (Fig. 14). Desmosomes, although few in number, were seen between the cells of the two types of epithelium (Fig. 14). Ciliated cells close to the metaplastic areas frequently lacked cilia, which appeared to be replaced by microvilli, but they could still be recognized by the presence of basal bodies. In goblet cells only a few mucous granules or remnants of granules could be recognized. Ciliated and goblet cells of the non-metaplastic epithelium frequently appeared to be pushed up by the deeper metaplastic cells. It is assumed that as the metaplastic epithelium expanded the non-metaplastic epithelium was sloughed off.

2. Squamous Metaplasia of the Urinary Bladder Epithelium of the Rat in Vitamin A Deficiency.

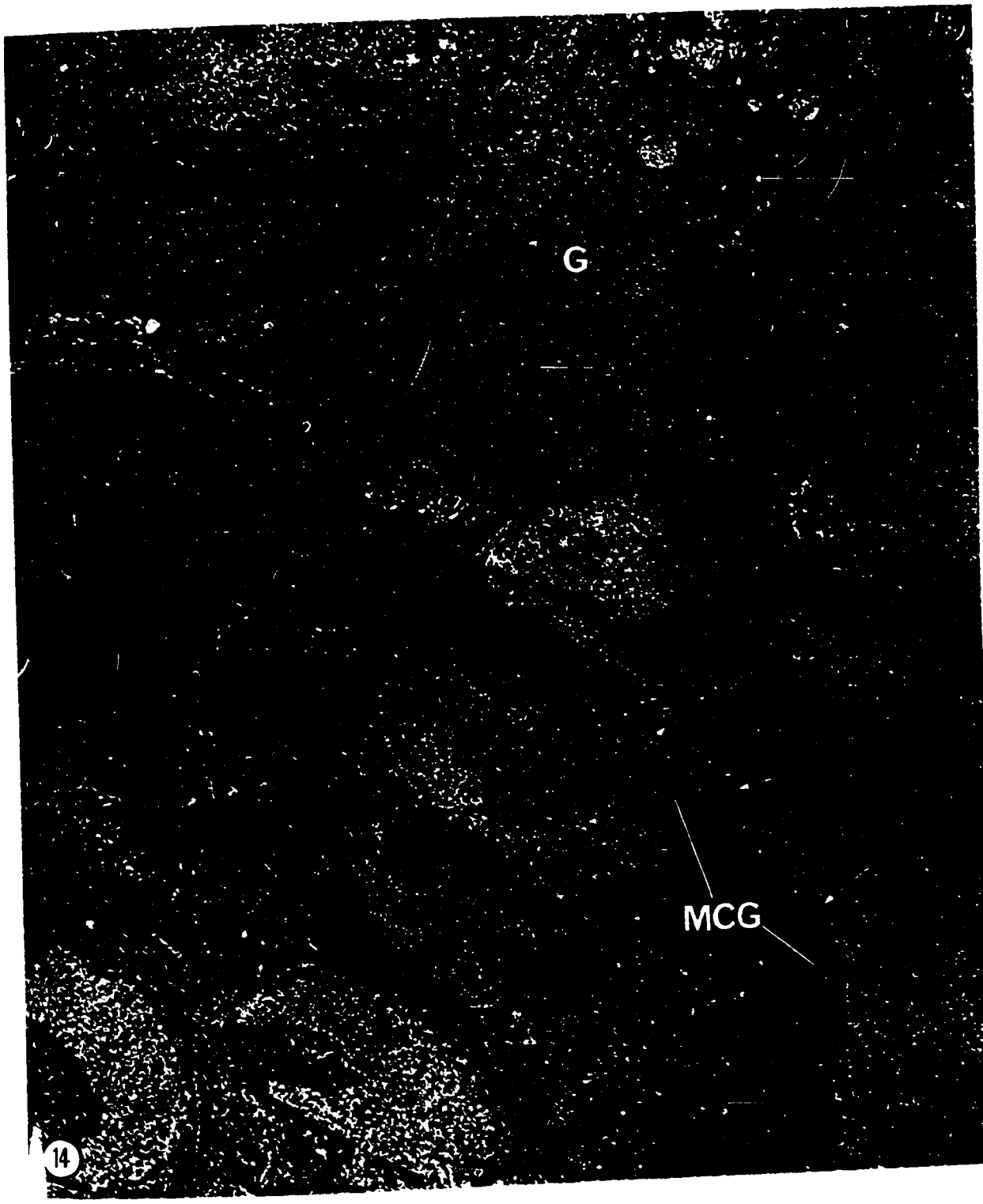
(a) Normal urinary bladder

The epithelial cells can be classified into squamous, intermediate, basal and a small number of bundle cells, with squamous cells covering the entire surface of the bladder. The detail of these cell types have been described by Hicks (1968). Therefore, only the points pertinent to the present investigation will be given here.

In agreement with the description given by Hicks (1968) the squamou cell was of variable shape and its free surface was characterized

Figure 14. Junction of the metaplastic (lower) and the non-metaplastic (upper) epithelium. Desmosomes are present along the border (arrows). The membrane-coating granules (MCG) are concentrated toward the border. G = goblet cell. 22-week vitamin A deficiency. Vestopal, UA and LC.

X 7300


G
MCG
14


MGC
14

by many oddly shaped, irregular projections (Fig. 15). The plasma membrane over the luminal surface of these cells was thick, measuring approximately 120A° (Fig. 16). It was composed of a thick outer component and a thin inner component. A similar asymmetric unit membrane (AUM) was seen to surround the numerous angular cytoplasmic vesicles (Fig. 16). The vesicles were either flattened and fusiform in shape or dilated and roughly spherical, but with angular conformation, as if the thick membrane was unusually rigid. The fusiform vesicles were most numerous towards the luminal border of the squamous cells, but were also seen throughout the cytoplasm. These vesicles also occurred, but in fewer numbers, in the cytoplasm of the intermediate cells just beneath the squamous cells. The remainder of the squamous cell was bounded by AUM. The lateral and basal plasma membrane of squamous cells was deeply convoluted (Fig. 16). These cells were held together by tight junctions.

The Golgi apparatus (GA) occupied a juxta-nuclear position near the base of the squamous cells. The cisternal membranes were of varying thickness in many cases. In places they had a usual (60A°) unit membrane, while in other places they were modified and thickened to over 100A°. The change was multifocal and not uniform throughout the GA.

A large number of membrane-bounded polymorphic structures (heterogeneous bodies) were regularly found in the squamous cells (Fig. 15).

Numerous tonofilaments were oriented primarily parallel to the cell surface, and most prominent in the apical half of the cell. The electron density of the squamous cells was lower than that of the intermediate cells, probably because of lack of well developed ER and few ribosomes.

Figure 15. Normal transitional epithelium of the rat. The squamous cell is characterized by a serrated luminal membrane and a large number of fusiform vesicles (FV) in the cytoplasm. Numerous tonofilaments (f) are oriented parallel to the surface. In addition, several membrane-bounded polymorphic structures (densely stained) are also shown. Durcupan, UA and LC.

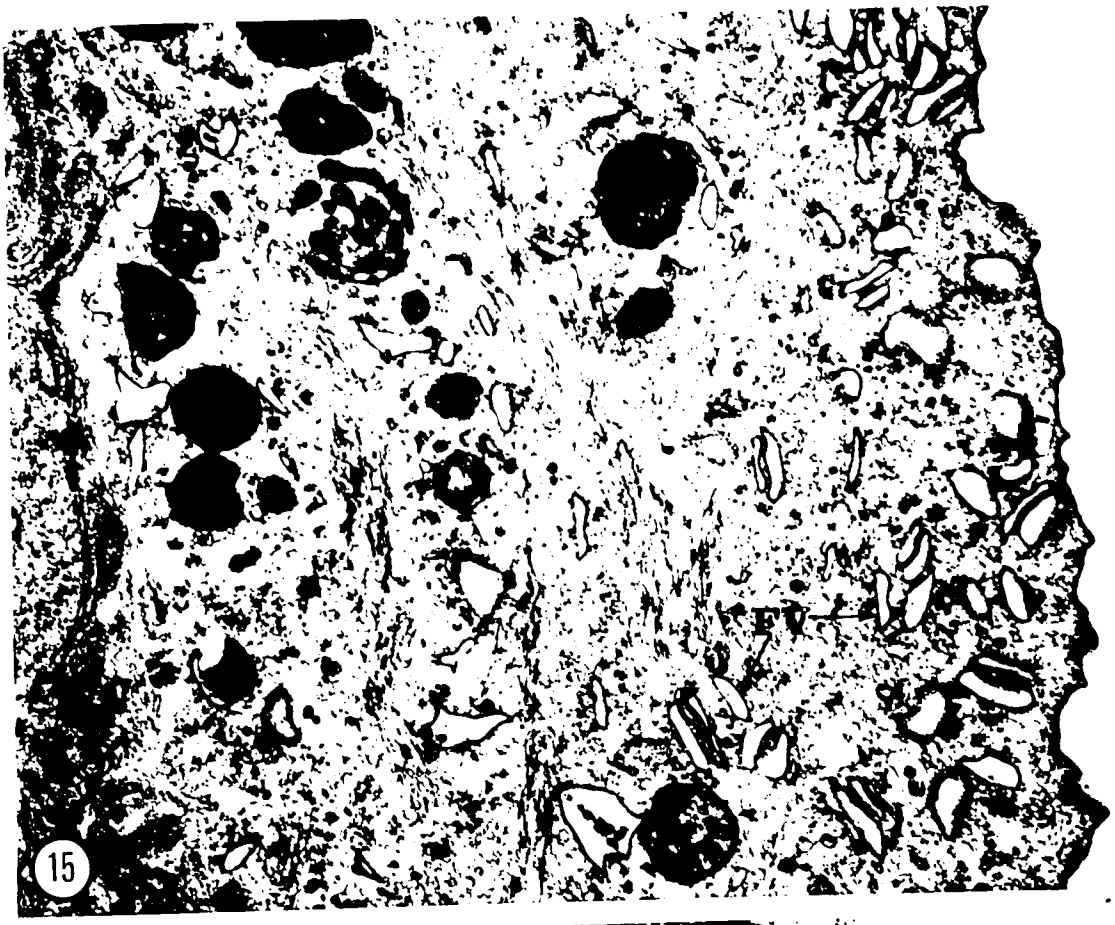
X 20,570

Figure 16. A higher magnification of a portion of two squamous cells. The membranes between the cells are highly convoluted and a tight junction (TJ) is seen near the lumen. The luminal surface of the squamous cells is bounded by asymmetric unit membranes (AUM). The outer dense lamella of the unit membrane is twice as thick as the inner dense lamella. Similar membrane is found bordering fusiform vesicles (FV). Durcupan, UA and LC.

X 89,280

15

16


FV
15


AUM
16

The intermediate cells were one or two layers in thickness between squamous and basal cells. They had higher cytoplasmic opacity and more prominent RER and ribosomes. Asymmetric unit membrane (AUM), vesicles, and heterogeneous bodies were seen in smaller numbers. The GA was prominent and regions of thickened cisternae were observed. Hicks (1968) and Koss (1969) found this only in the GA of the squamous cell.

The basal cells were either cuboidal or columnar. They rested on a thin basement membrane and had comparable opacity to intermediate cells.

Bundle cells were first described by Hicks (1965) in rat ureter, and later by Monis and Zambrano (1968a) in rat bladder. The cytoplasm contained large vacuoles, irregular in shape and surrounded by thin membranes which were absent in areas. The vacuoles contained dense material and bundles of filaments.

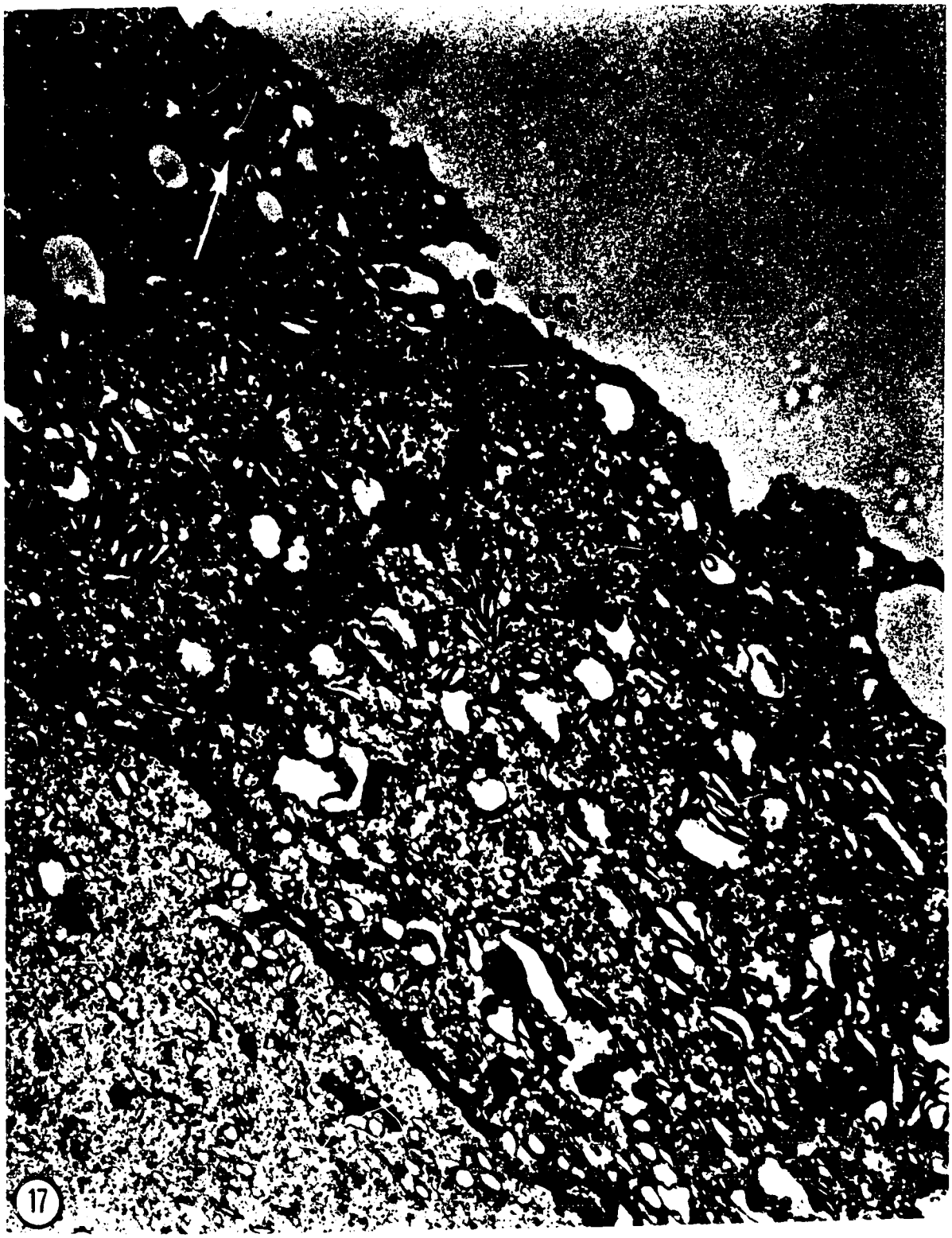
Ruthenium red tagged very heavily the luminal surface coat and also some vesicles in the cytoplasm (Fig. 17). The majority of the vesicles were not "stained" by ruthenium red. At higher magnification the cell coat of the squamous cells (and also the tagged vesicles) was made up of filamentous material (Fig. 18). The thickness of this fuzzy coat was variable. The cell coat of intermediate cells was sometimes stained.

(b) Bladder epithelium of vitamin A deficient rats.

A very varied response of the bladder epithelium to vitamin A deficiency was observed. Even in a single animal the changes were often localized. Cornified patches were found in animals after one to 22 weeks on vitamin A deficient diet. The epithelial response in any one animal was also varied from simple hyperplasia to the formation of

Figure 17. Normal rat urinary bladder epithelium treated with ruthenium red. The cell coat (CC) material of the squamous cells (S) is densely stained. With the exception of the few vesicles (arrows). The fusiform vesicles are not stained by ruthenium red. Durcupan, UA.

X 13,440



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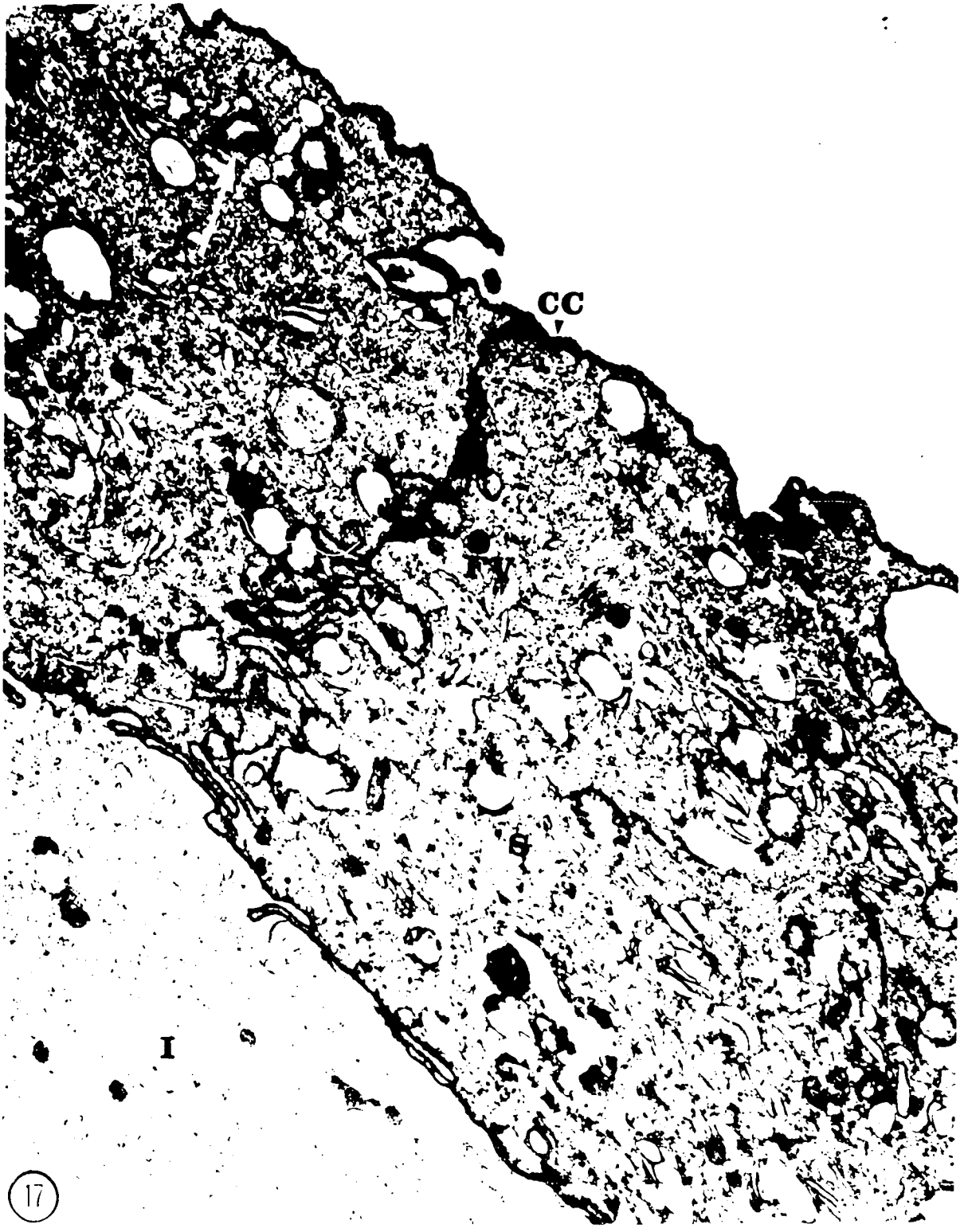


17

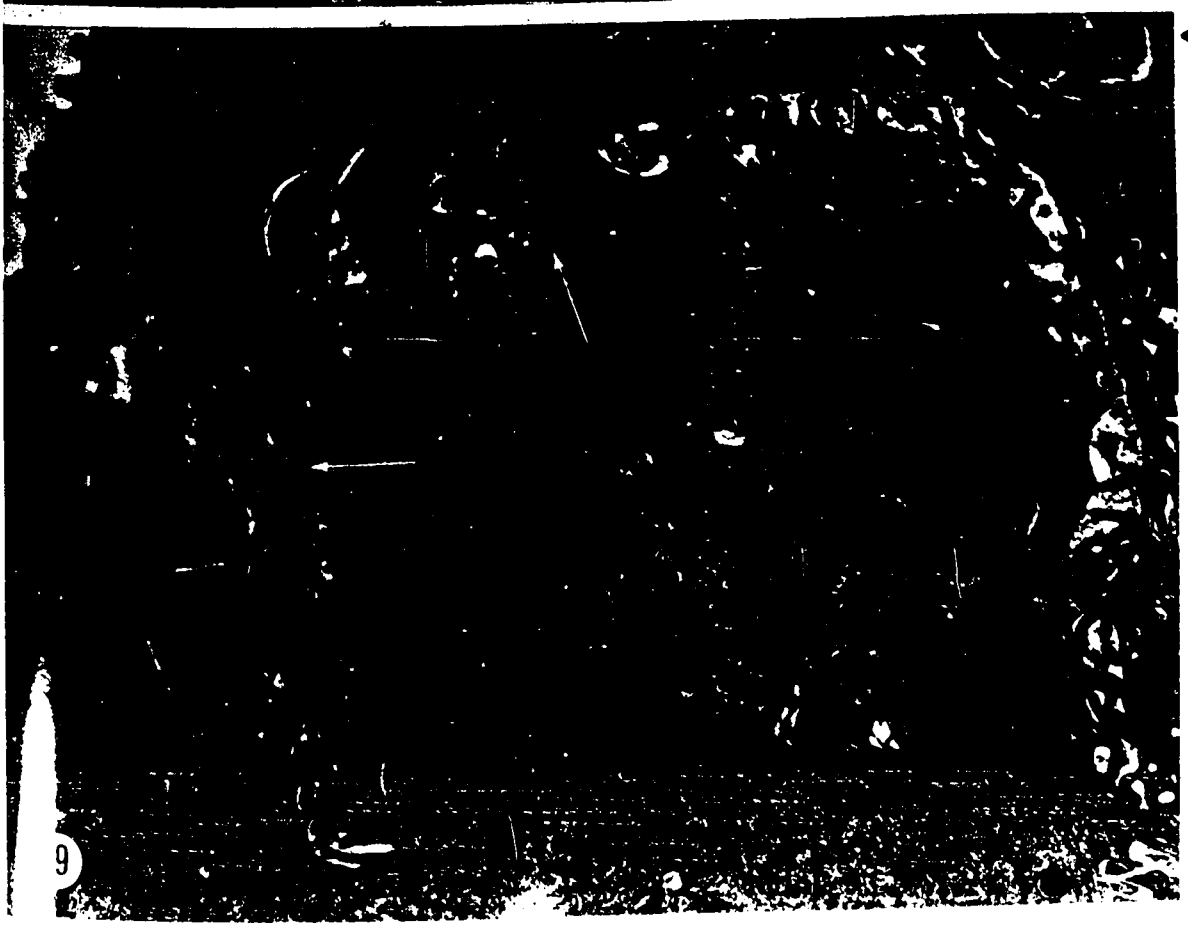
Figure 18. At high magnification, the cell coat (CC) of the squamous cells is made up of filamentous material. Durcupan, UA and LC.

X 43,830

Figure 19. Portion of hyperplastic rat urinary bladder epithelium. The intercellular spaces are wide. The fusiform vesicles (arrows) are all slit-like, with very narrow lumen or no lumen at all. 21-week vitamin A deficiency. Vestopal, UA and LC.

X 20,550


18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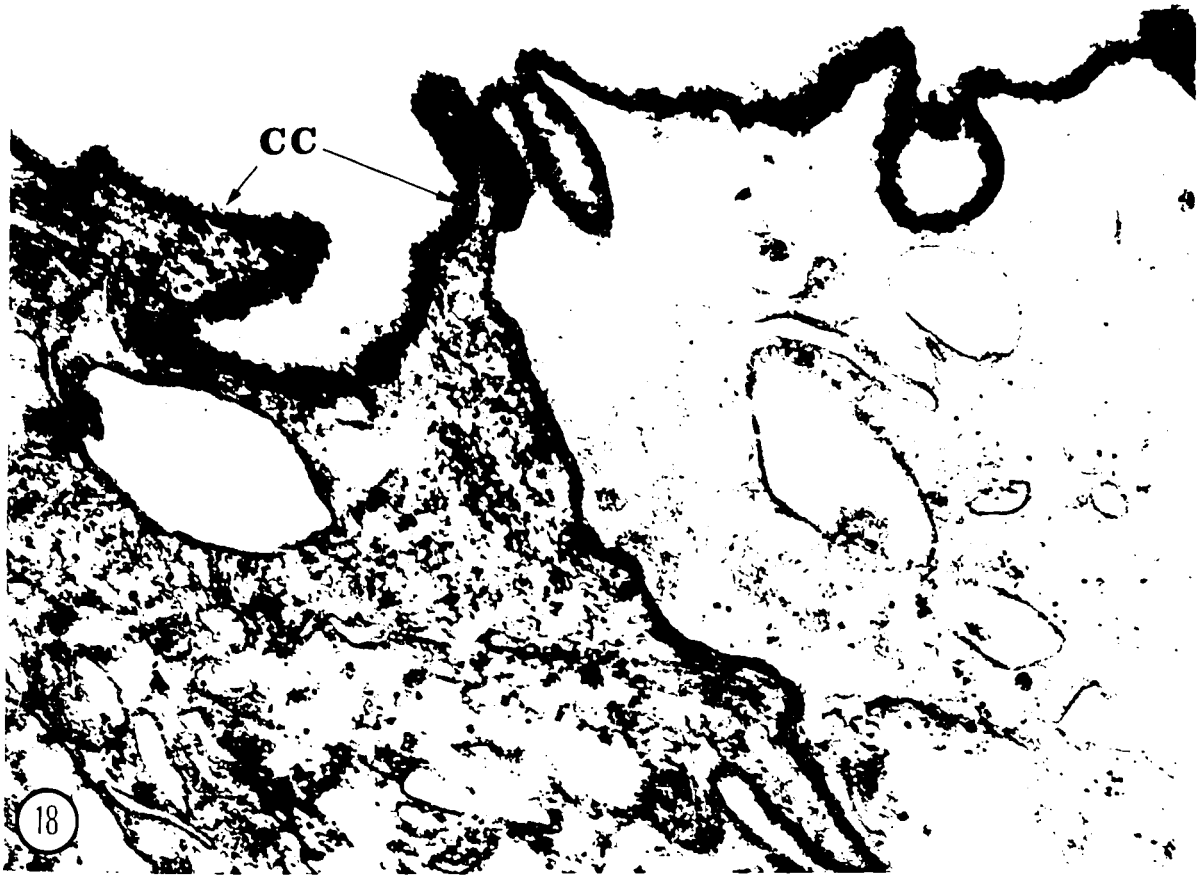
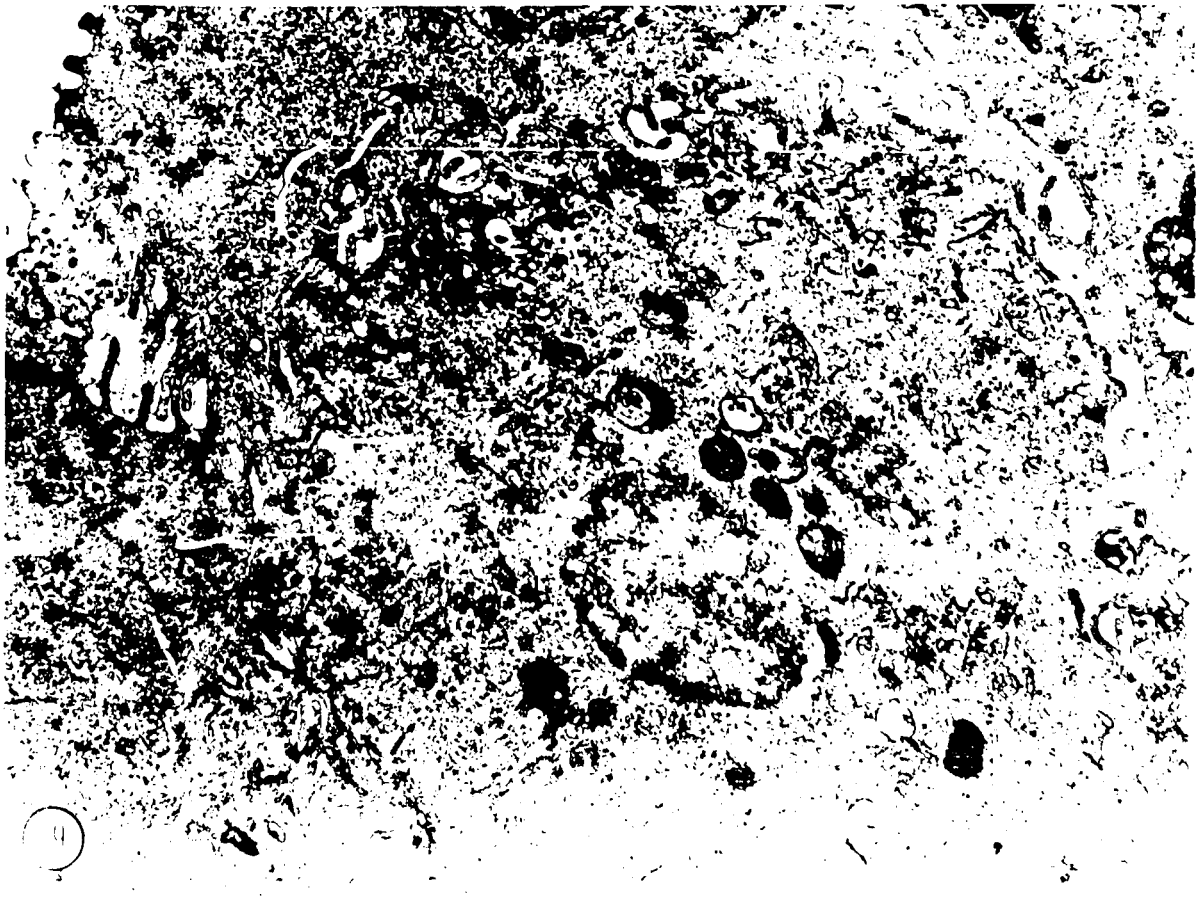

CC
18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fully cornified stratified epithelium. The structure of these areas is described below.

(i) Simple hyperplasia

These areas were structurally similar to the normal control ones in various cell layers, except that the intermediate cells increased from one or two to many layers.

Another slightly different type of hyperplasia was observed (Fig. 19). It differed from the former in at least three aspects: (i). The vesicles were slit-like or collapsed. In many cases lumens were not perceivable. (ii). The vesicles were present in intermediate cells deep into the layer. (iii). Similar cytoplasmic density was observed in squamous and intermediate cells. Although ribosomes were abundant in these cells, aggregates that looked like keratohyalin granules (KH) were not observed. Granular hyperplasia as reported by Hicks (1968) was not found.

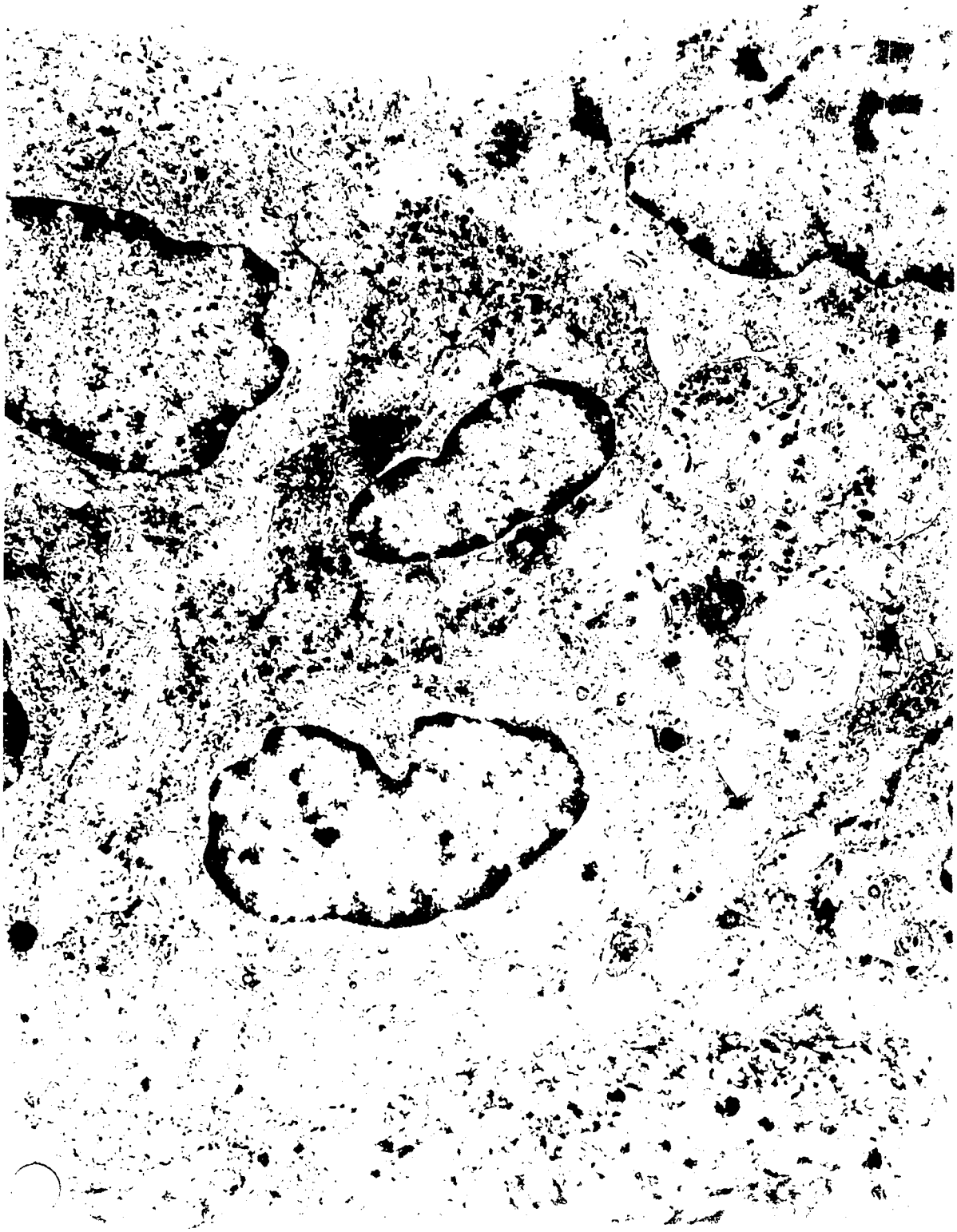
(ii) Semi-cornified metaplasia.

Various types of semi-cornification have been observed. There was one feature common to them. This was, that, with the exception of the superficial three , four or more layers of cells, all the other cell layers contained large amounts of keratin fibrils and resembled the basal and intermediate cell layers of the cornified epithelium (Fig. 20). The MCG were very prominent in the layer of cells at the junction or one to two layers beneath it. The intercellular spaces of both superficial cells and keratinocytes were wide and cytoplasmic projections intermingled with adjacent cells, some of them terminating with desmosomes. The transition between the keratinized "base" and the non-keratinized superficial cells was abrupt. Desmosomes were found along the border.

Figure 20. Portion of semi-cornified epithelium, with the cornifying (C) epithelium at the bottom and non-cornifying (NC) epithelium at the top. The boundary between the two kinds of cells is indicated by arrows. A large number of keratin fibrils can be seen in the cornifying epithelium, while in the non-cornifying epithelium relatively few of them are present. Typical fusiform vesicles are not seen in this particular region. Instead, modified vesicles, with dense luminal contents can be seen, the detail of which is presented in Figures 22 and 23. 19-week vitamin A deficiency. Vestopal, UA and LC.

X 13,440


NC
C
C
20



At some areas the superficial cells remained normal and showed typical characteristics of bladder epithelium. But in other areas various changes within these cells were observed. The latter will be described in the following paragraphs.

The first variant of superficial cells of semi-cornified epithelium was the reduction or total absence of cytoplasmic angular vesicles (Fig. 21).

The second variant was characterized by the presence of a peculiar type (Type I) of vesicle (Figs. 22, 23). The vesicles were of similar shape and size to those of the normal. The membrane was asymmetrical and had a measurement of 120A° thick. The membrane was rigid and usually showed angular conformation.

Unlike the normal there was granular dense material in the lumens of these vesicles. The dense material filled the vesicles in such a way that a "halo" could always be seen between the dense material and the thick vesicular membrane (Figs. 22, 23). There was more dense material in some vesicles than in the others.

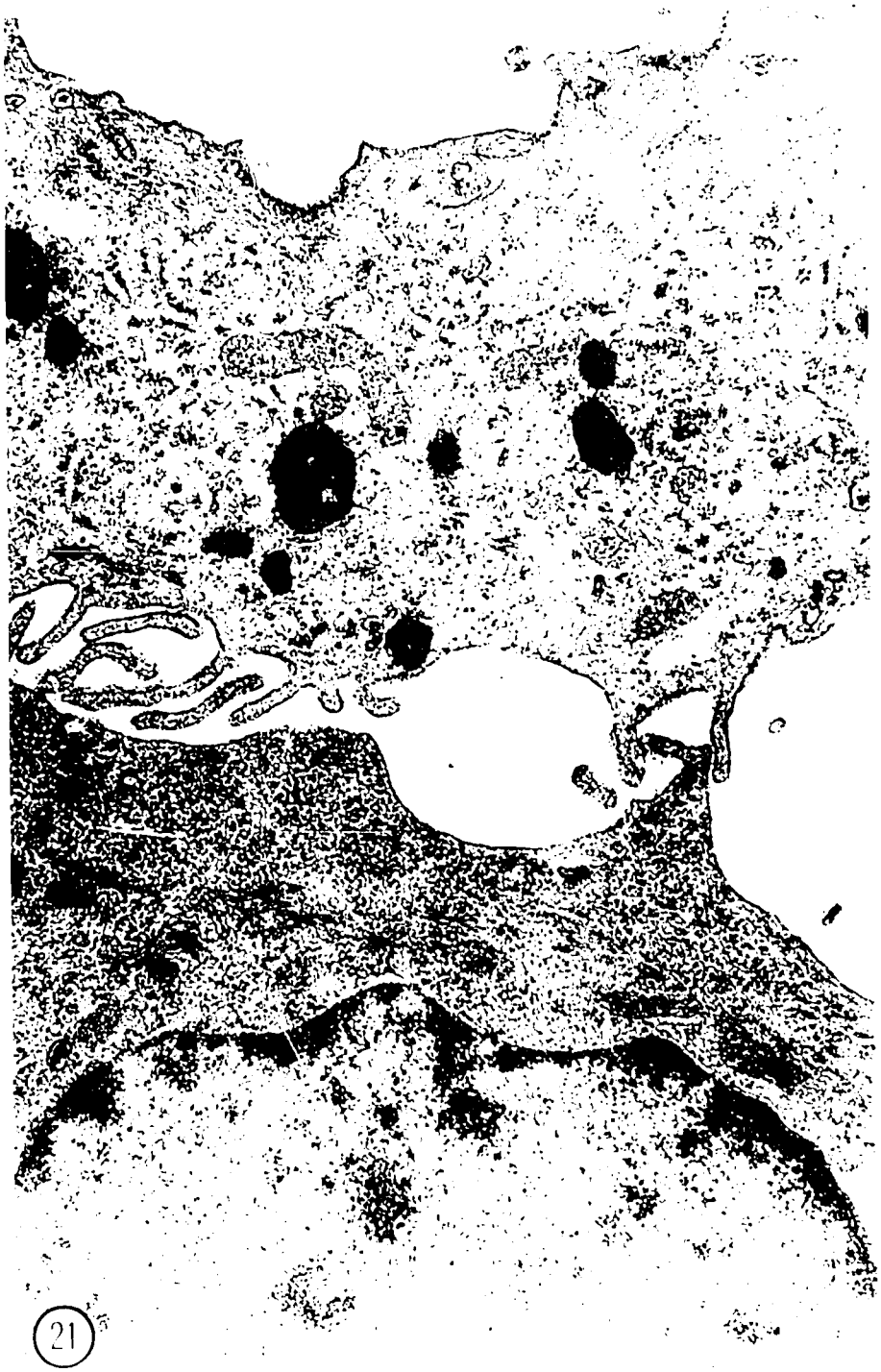
A second type (Type II) of vesicle (or granule) possessed by the superficial cells of some areas is shown in Figures 22 and 24. These granules were oval or spherical, quite uniform in size, measuring approximately 140 mμ in diameter, which was comparable to the size of MCG. Unlike the MCG these granules were bounded by a distinct AUM (Figs. 22, 24), similar (but thinner, measuring about 100A°) to that of the vesicles or luminal membrane of the bladder. The "halo" was less distinct and in many cases could not be recognized. Unlike the MCG the matrix of these vesicles was not laminated.

MCG have not been observed in the superficial vesicle-bearing cells. Although some granules had the appearance of MCG and were

Figure 21. Another region of non-cornifying epithelium. There are practically no fusiform vesicles in the cells. 19-week vitamin A deficiency. Vestopal, UA and LC.

X 25,200

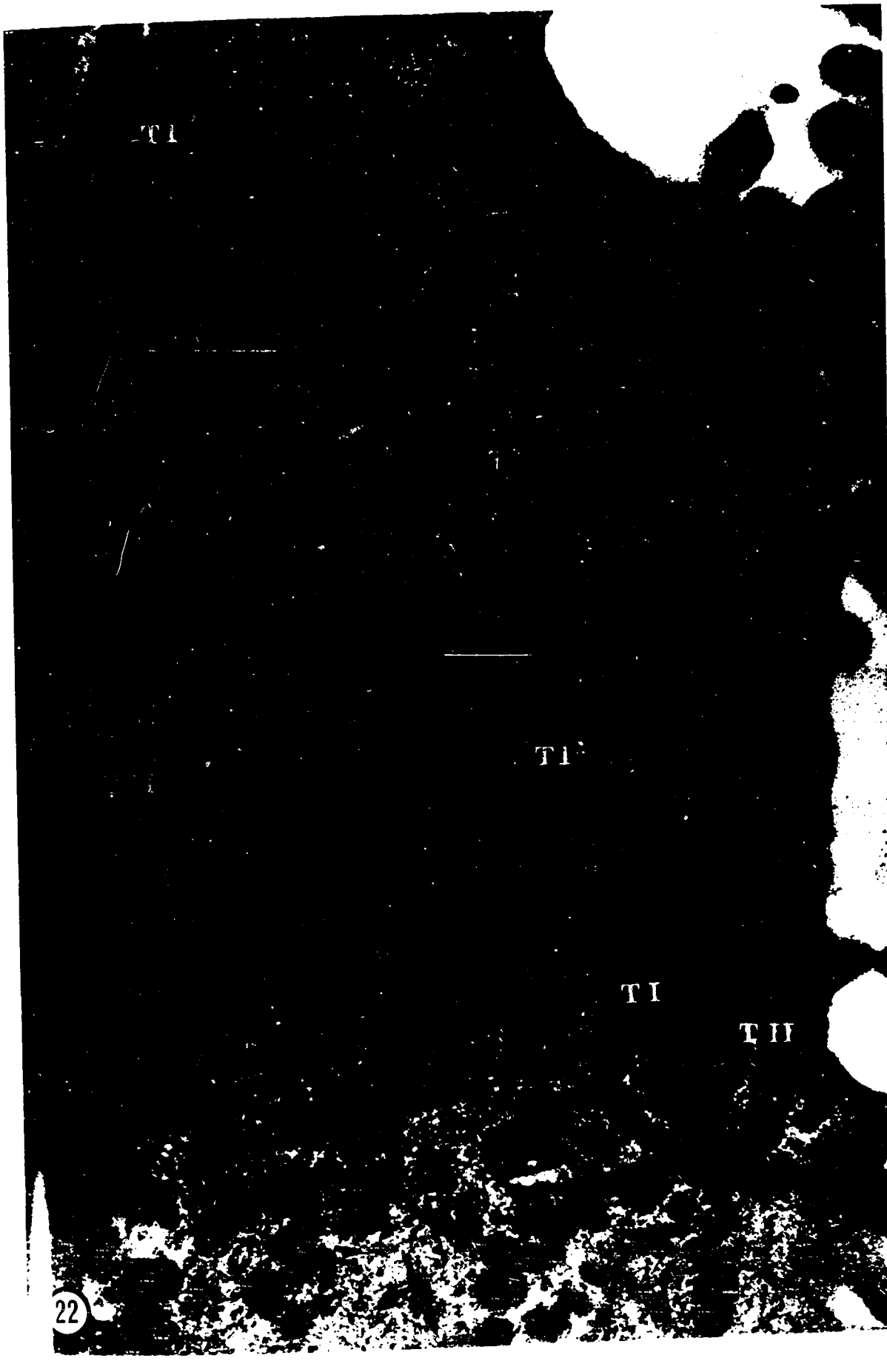

21



21

Figure 22. The modified vesicles (type I and II) of semi-cornified bladder epithelium. The type I vesicles are of similar shape and size to those that of the normal bladder. The AUM is still prominent. Unlike the normal, there is granular dense material in the lumens of these vesicles. The type II vesicles are usually round or ovoid and have less distinct AUM. In addition, some fibrillary material (f) is also shown in the background. 19-week vitamin A deficiency. Vestopal, UA and LC.

X 80,000


TI
TI
TI
TII
22

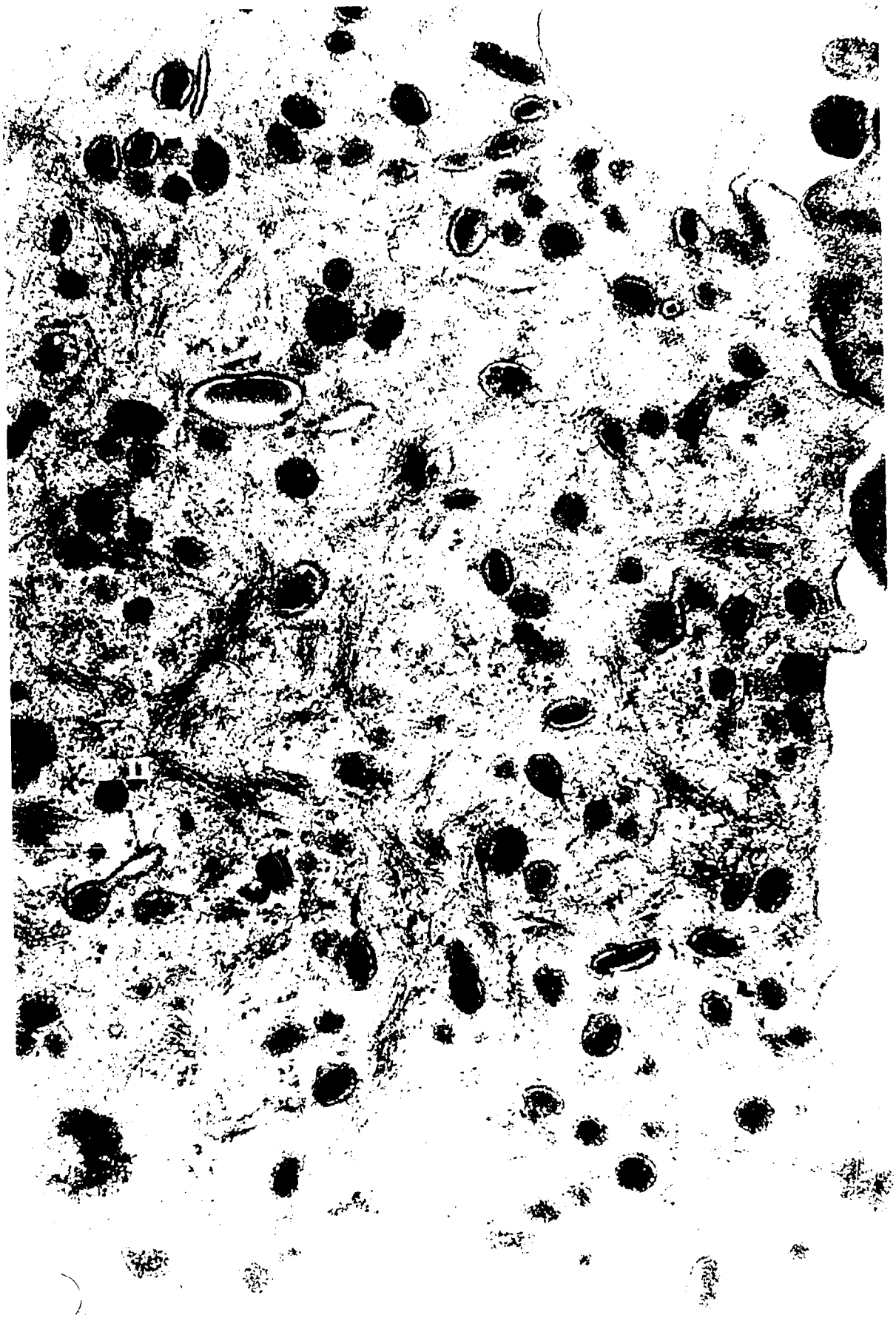
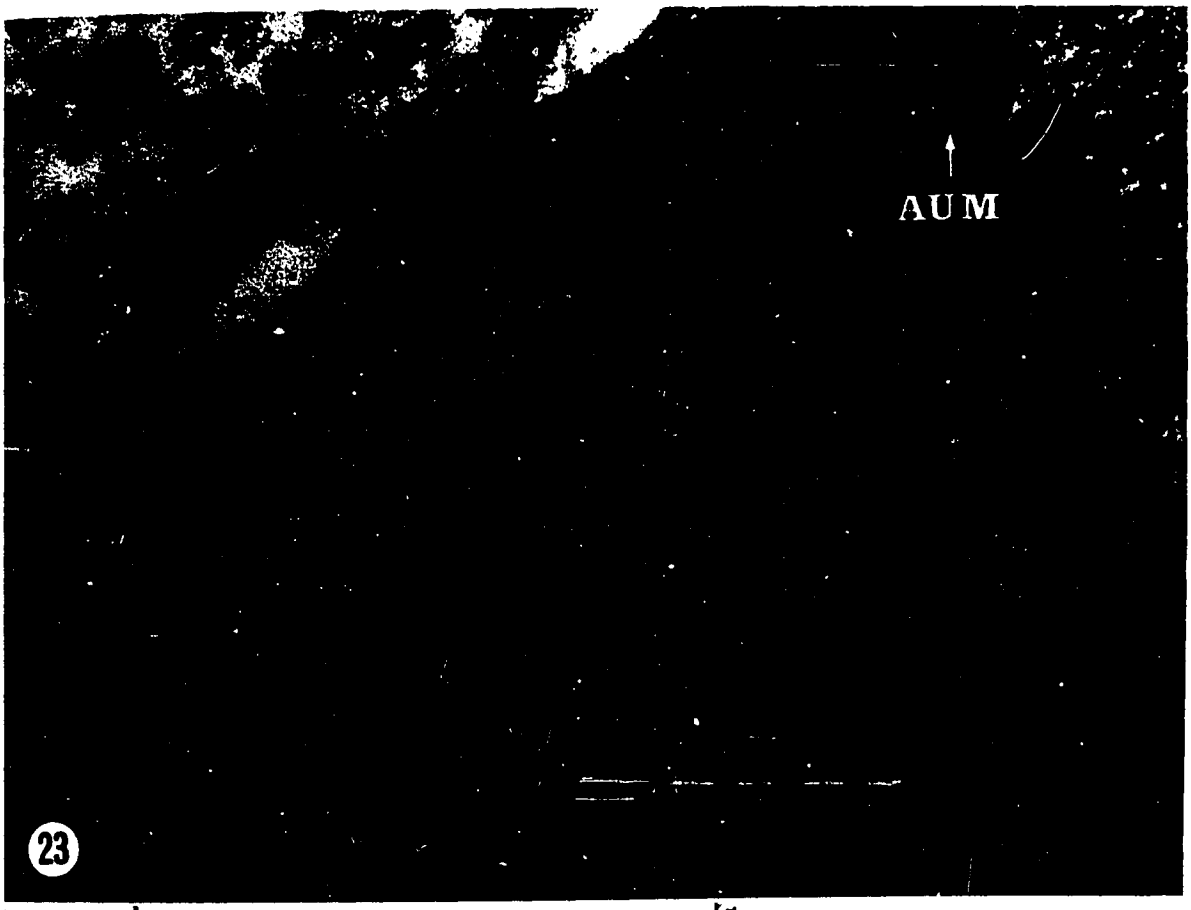


Figure 23. A higher magnification picture showing the type I modified vesicles. The dense material filled the vesicles in such a way that a "halo" could be seen between the dense material and the asymmetric vesicular membrane (AUM). 19-week vitamin A deficiency. Vestopal, UA and LC.

X 100,000

Figure 24. The type II modified vesicles. They are more regular in size (140mm) and shape then type I vesciles. They are bounded by asymmetric membranes (although thinner than those of type I). The halo (arrows) of the vesicles is less distinct. 19-week vitamin A deficiency. Vestopal, UA and LC.

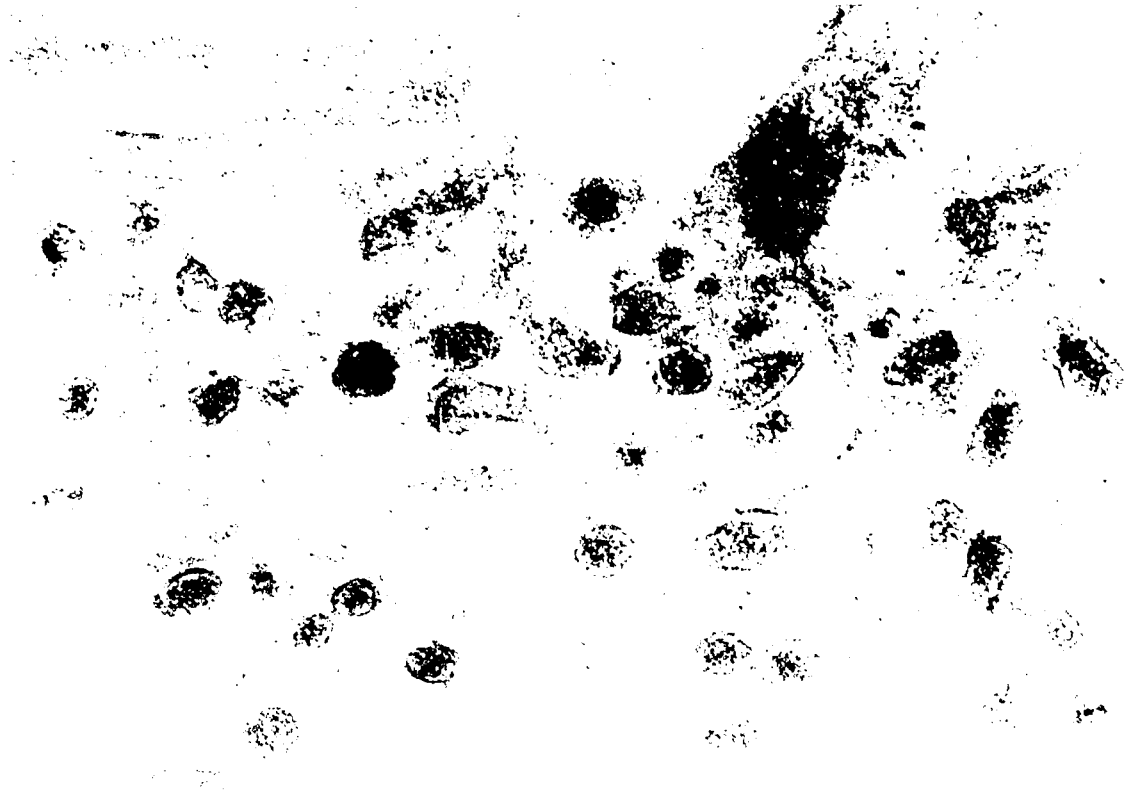
X 77000



23

24


13



bordered by conventional unit membrane, laminations of matrix have not been observed, so that we cannot be sure whether these were truly MCG or just another variant of the above mentioned vesicles.

In some superficial cells masses of dark granular matrix were observed among the modified vesicles (Fig. 25). They appeared to be formed from aggregates of ribonucleoprotein particles. They were not membrane bounded, and were quite similar to KH granules as described by Hicks (1968) in granular hyperplasia of rat bladder.

The GA were prominent in many of the superficial cells and focal specialization of Golgi membranes has been observed in all layers of superficial cells. Aside from desmosomes, tight junctions were commonly seen between cells (Fig. 26). Vesicles, both normal and modified were found also in the cytoplasmic projections of these cells. The vesicles and granules of superficial cells were mostly distributed toward the luminal side. The content of fibrillary material was higher than in the normal (Fig. 22), but there were no ogranized fibrils. Heterogenous bodies remained the prominent feature of these cells. AUM of luminal membrane was less prominent in the superficial cells. On the other hand, AUM have been observed in regions other than the luminal surface.

Bundle cells have also been observed in cornified and semi-cornified epithelium.

(iii) Cornified epithelium

In the cornified areas, four morphologically distinct layers (basal, spinous, granular and cornified or horny layers) were observed (Fig. 27). The cuboidal basal cells were structurally very similar to basal cells of normal animals. The cytoplasmic filaments in these cells

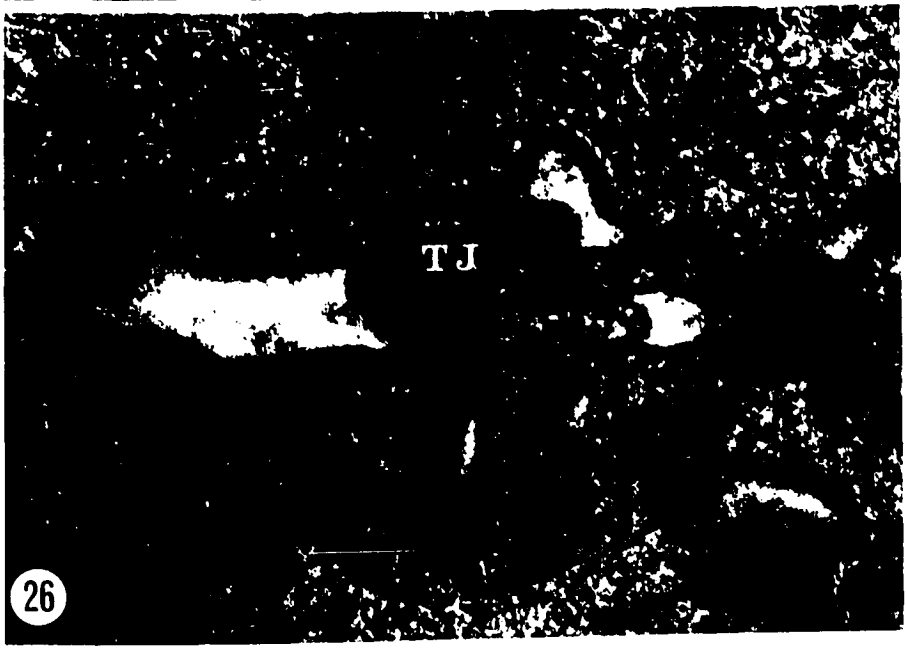
Figure 25. Field showing the presence of keratohyalin (KH) granules in the superficial non-cornifying cells of the semi-cornified epithelium. Many modified vesicles (v) (type II) are also present. 19-week vitamin A deficiency. Vestopal, UA and LC.

X 80,000

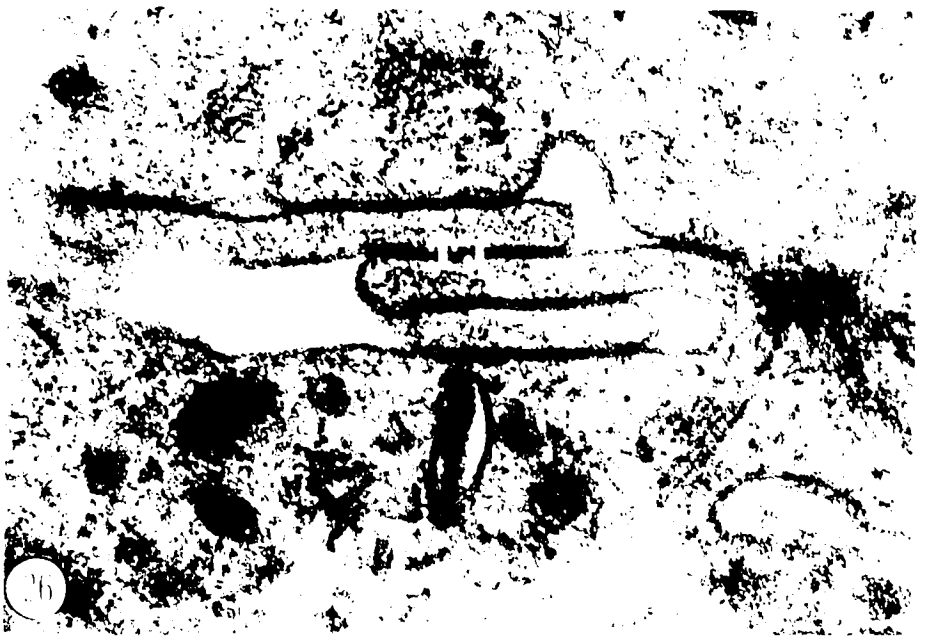
Figure 26. A series of four tight junctions (TJ) between adjacent bladder epithelial cells is shown. 19-week vitamin A deficiency. Vestopal, UA and LC.

X 80,000


D
KH
25


TJ
26

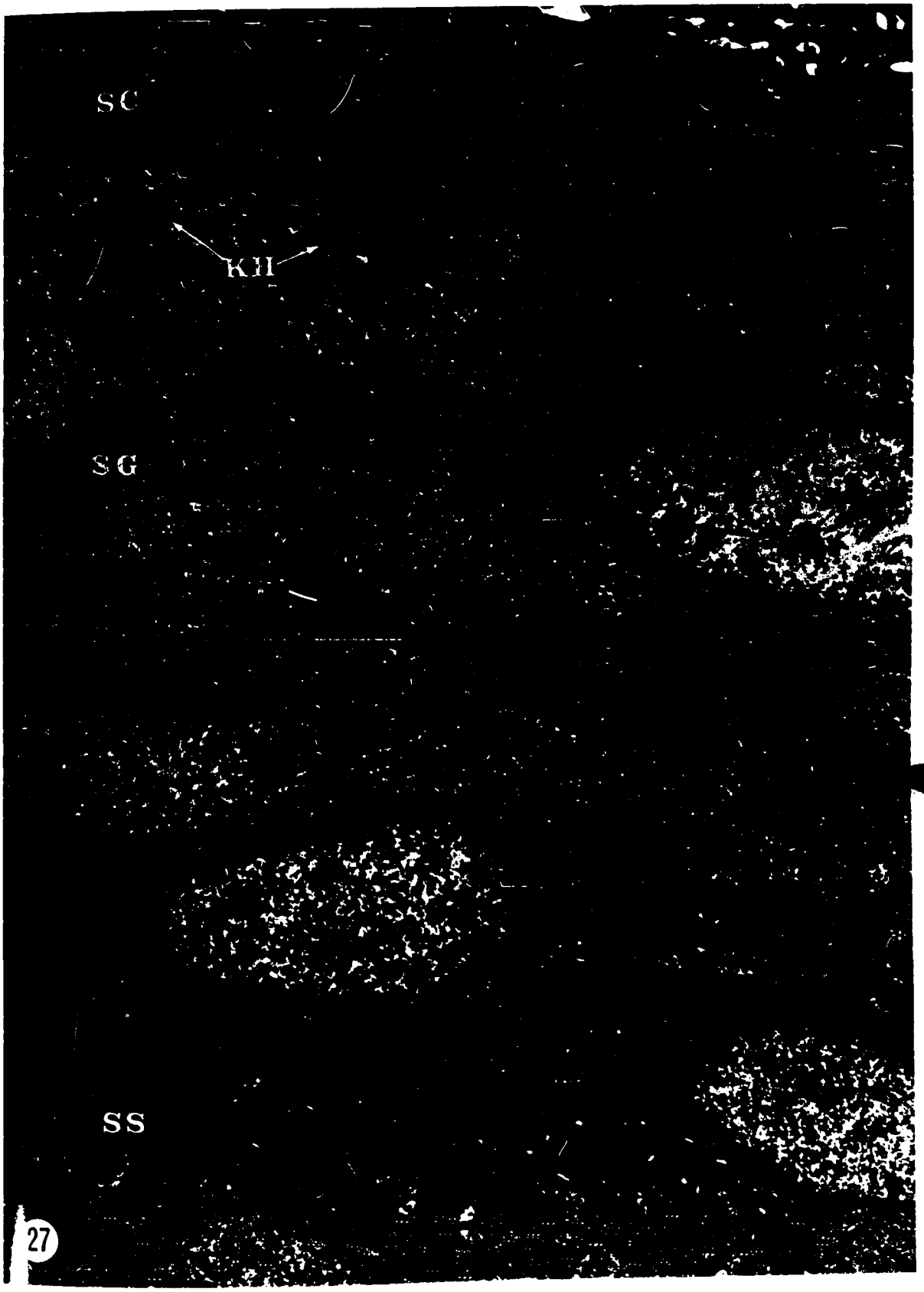
25

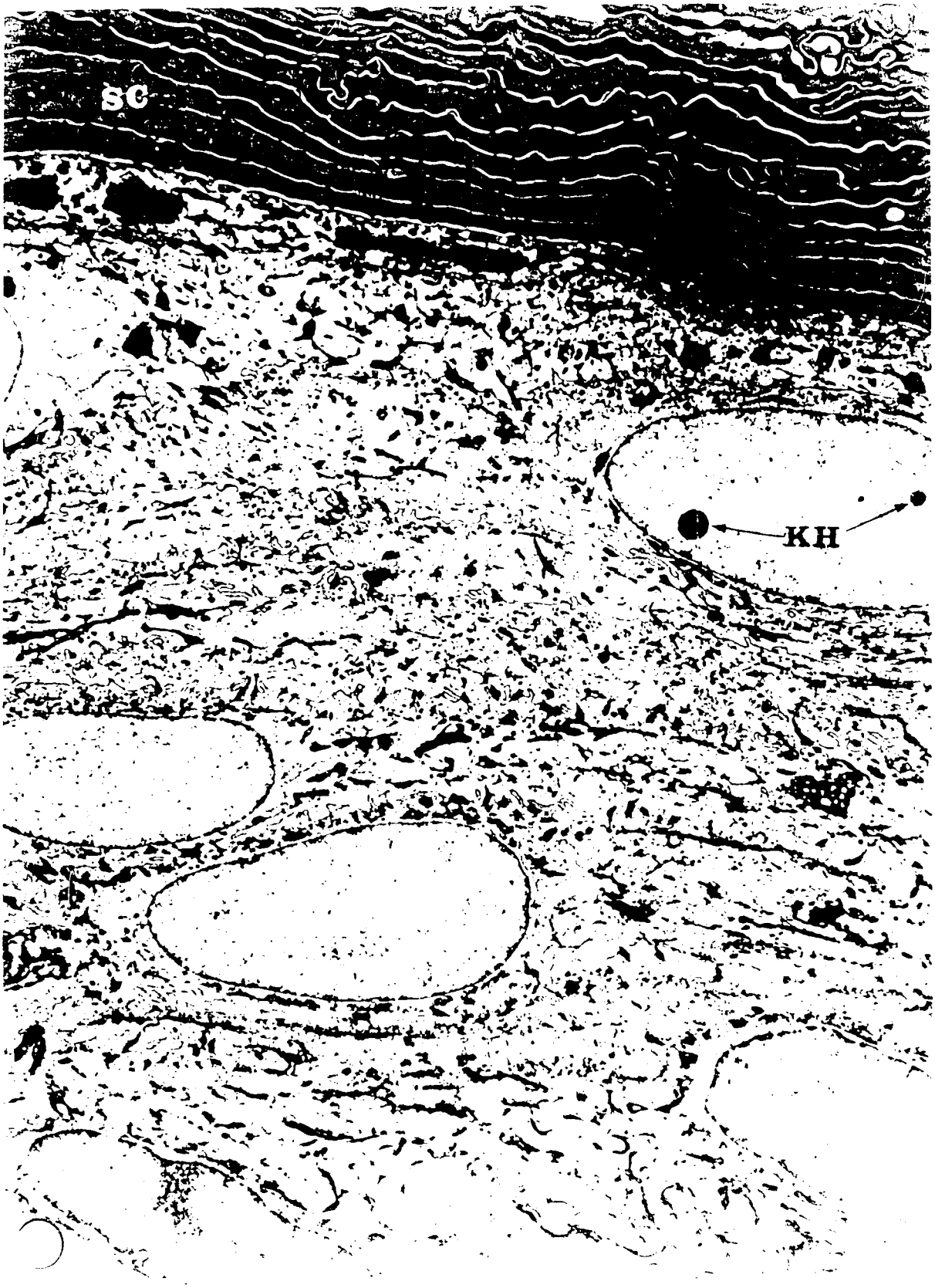


26

Figure 27. A low magnification picture of cornified bladder epithelium in vitamin A deficiency. The stratum corneum (SC) is very thick and composed of densely stained horny cells. The stratum granulosum (SG) is characterized by the presence of keratohyalin granules, both in the cytoplasm and in the nucleus. The cytoplasm is packed with keratin fibrils, while other organelles are less prominent. Portions of stratum spinosum (SS) is shown at the bottom of the picture. 17-week vitamin A deficiency. Vestopal, UA and LC.

X 6,560


SC
KH
SG
SS
27


SC
KH

were aggregated to form fibrils and were much more abundant than in the controls. Ribosomes or ribonucleoprotein particles were packed in the cytoplasm.

Half-desmosomes and desmosomes appeared better developed and more numerous than in the normal.

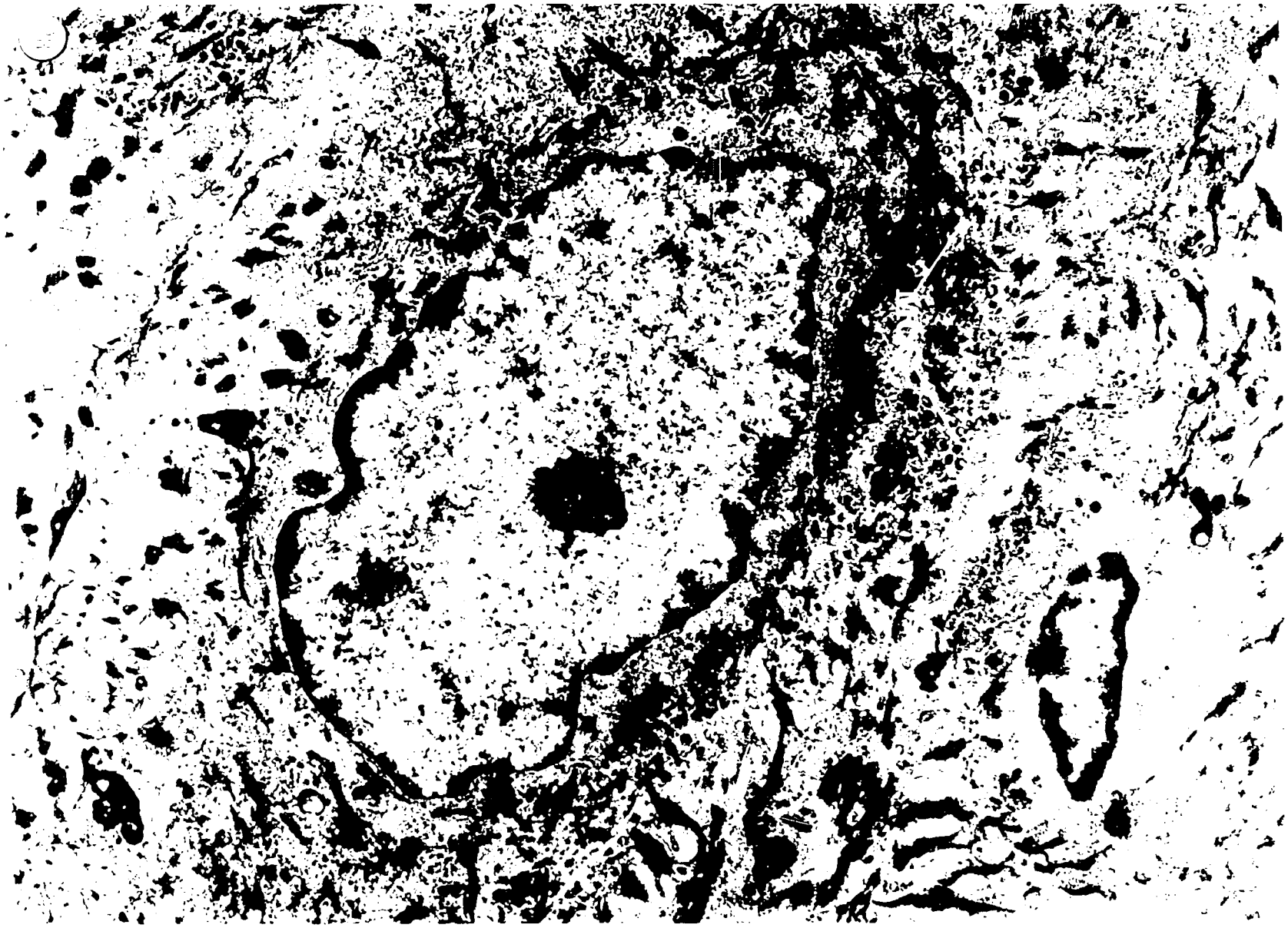
Langerhans cells, though not observed in the normal animals, were present in small numbers between the basal cells and also the intermediate cells. The details of these cells will be reported under a separate section.

The spinous cells (Fig. 28) varied from two to five or six layers thick. The cells nearest the basal cells were stellate in shape. The successive ones, before the granular layer was reached, were more flattened and elongated. The cells were connected by numerous cell processes and desmosomes were similar to the spinous cells in the epidermis (Brody, 1959; Motaltsy and Parakkal, 1967; Odland and Reed, 1967). The cytoplasmic filaments were much more abundant than in the basal cells. They enveloped the nucleus of the spinous cells (Fig. 28). The fibrils extended into the cell processes and terminated by desmosomes. A typical desmosome, as described by Farquhar and Palade (1963), consisted of attachment plaques associated with the inner dense lamellae of the trilaminar unit membrane (Fig. 29) at directly opposite areas of adjacent cells and into these areas tonofilaments were embedded. An intercellular disc lay between the attachment plaques parallel to the plane of the adjacent cells (Fig. 29). The intercellular desmosomal disc was separated from the dense attachment plaques by two thin gaps, one on each side. These thin gaps could be traced to be continuous with the central light lamella of the unit membrane (Fig. 29).

Figure 28. The field shows a section through the spinous or prickle cell layer of a cornified area of bladder epithelium. The edges of the prickle cells are drawn out into long processes which interdigitate with those of adjacent cells in the extracellular space. The nucleus is big and has a prominent nucleolus. A large number of keratin fibrils is present in the cytoplasm, which lies immediately adjacent to the nucleus of the prickle cells. Membrane-coating granules (MCG) can also be identified in this picture. 17-week vitamin A deficiency. Vestopal, UA and LC.

X 28,050


MCG



Tight junctions, which are normally found in cells bordering the lumen were found between spinous cells. The rest of the cytoplasm was packed by ribosomes and other organelles of the cells. In the cells just below the granular layer, distinct granules, (Figs. 28, 30) similar to the membrane-coating granules (MCG) of the skin (Odland, 1960; Matoltsy and Parakkal, 1965, 1967) were observed. They were more numerous near the luminal side of the cells. The granules were ovoids or short rods and measured 150 mμ in width. At high magnification they were seen to be laminated (Fig. 30). Some small keratohyalin (KH) granules were observed in the spinous cells.

The granular layer was three or four cells in thickness. The most characteristic feature was the presence of large numbers of KH granules (Figs. 27, 31). They were irregular in shape and varied in size. They were found not only in the cytoplasm but also in the nucleus (Fig. 27). Some of them were associated with the fibrils, while many of them lay free in the cytoplasm (Fig. 31). At high magnification they appeared as dense homogenous or granular masses in close contact with fibrils or ribosomes. It was of interest to note that the KH granules were of two morphological types. One appeared granular (Type 1), the other dense and homogeneous (Type 2). The former were bigger, irregular in shape, and resembled masses of aggregated ribosomes. The latter were usually round and either lay free in the cytoplasm or were associated with either keratin fibrils or with the granular form of KH granules and ribosomes (Fig. 31). The KH granules found in the nuclei were of dense type. Coalescence of KH granules to form bigger masses was observed in granular cells next to cornified (horny) cells (Fig. 27). Occasionally cells were seen filled with large masses of KH granules. Keratin fibrils were very abundant

Figure 29. The picture shows a typical desmosome of the cornified bladder epithelium. Dense attachment plaques (one indicated by an arrow) are associated with the inner dense lamella of the unit membrane of adjacent cells. An intercellular (or interdesmosomal) disc (►◄) is located between the attachment plaques. The interdesmosomal disc is separated by thin gaps, one on each side, from the dense attachment plaques. These thin gaps are continuous with the central light lamellae of the unit membranes of the adjacent cells. That is, the interdesmosomal disc is associated with outer lamellae of the adjacent unit membranes. 17-week vitamin A deficiency. Vestopal, UA and LC.

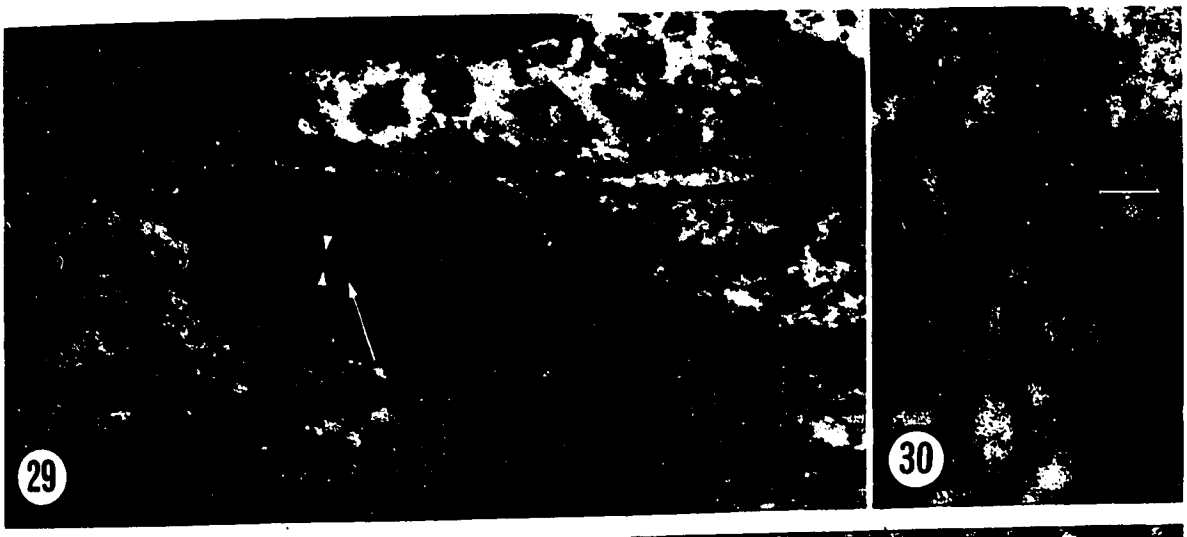
X 102,500

Figure 30. A typical membrane-coating granule of cornified epithelium is shown. It is about 150mμ in diameter and bears characteristic central laminations. 19-week vitamin A deficiency. Vestopal, UA and LC.

X 128,000

Figure 31. Electron micrograph showing two morphologically distinct types of keratohyalin granules from stratum granulosum of the cornified bladder epithelium. The first type (T1) is larger, granular and usually irregular in shape. They are found only in cytoplasm. The second type (T2) is smaller, dense and usually round in shape. They are found not only in the cytoplasm but also in the nucleus. Quite often they are found to associate with the granular type of KH granules. Both types of KH granules are closely related to ribosomes (arrows). 17-week vitamin A deficiency. Vestopal, UA and LC.

X 56,000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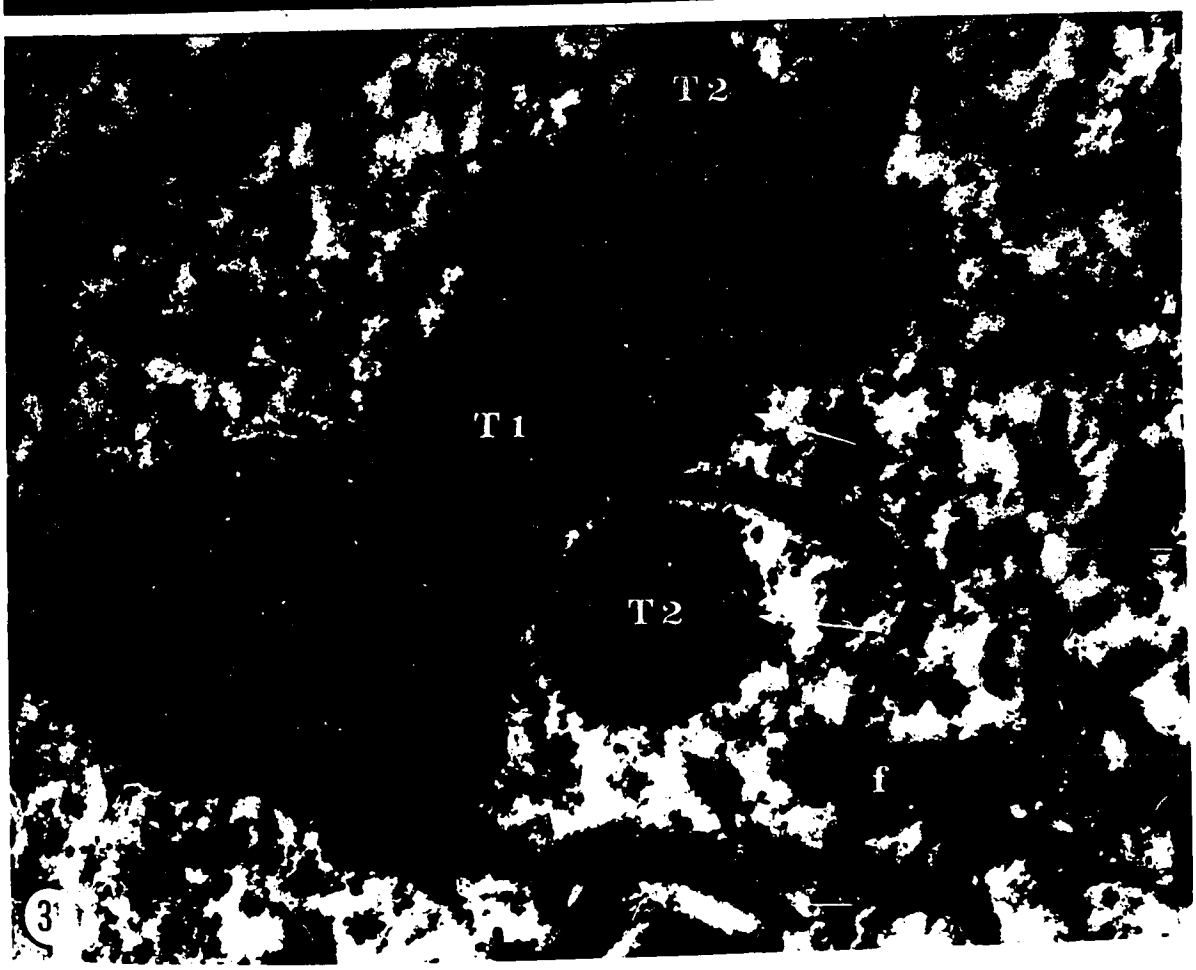

T2
T1
T2
f
31

29

30

T2
T1
T2
f
31

and most of the cell's organelles were lost.

MCG which first appeared at spinous cells became very prominent in this layer. They were distributed on the luminal side of the cells. Granules of similar appearance also have been observed in the intercellular spaces (Figs. 32,33). The discharge of these granules into intercellular spaces was suggested by this appearance.

The plasma membrane of the granular cells increased in thickness progressively, from about 70A° in the spinous cells to about 180A° in the granular cells just next to the horny cells (Figs. 32,33). Cell membranes in the granular layer were relatively straight, and studded with modified desmosomes. As the plasma membrane increased in thickness in the course of differentiation, the attachment plaques of the desmosomes began to merge into the thickened inner lamella of the plasma membrane. The intercellular desmosomal disc appeared to be "detached" from the original attachment plaques (Figs. 32,33).

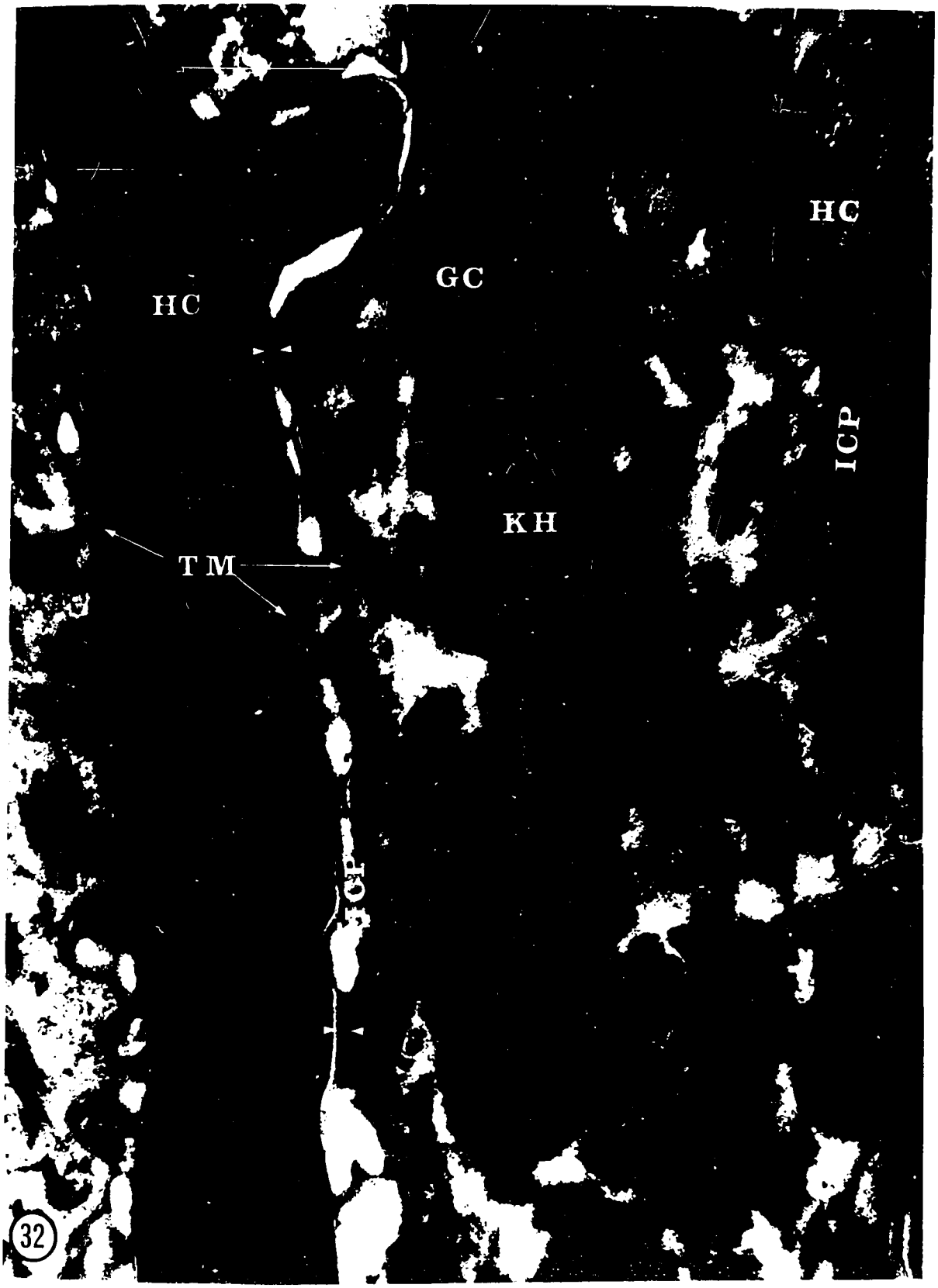
The cornified cells ranged from two or three to many layers (Figs. 27, 34). The cells were dense and most of the organelles seen in the other cells were not observed. However, discrete fibrils and filaments were present in some regions as well as KH granules and remnants of nuclei. At low magnification horny cells of different density could be seen at various levels, as has been described in the stratum corneum of the epidermis (Brody, 1970).

The plasma membrane of the cornified cells measuring approximately 200A° in thickness (Figs. 32, 34), was much thicker than that of the unit membrane.

Desmosomes underwent further modification and the attachment plaques were completely merged into the thickened membrane (Fig. 32).

Figure 32. Field showing a region at the stratum granulosum and corneum. Two horny cells (HC), one on each side of the picture, are separated by a central transforming granular cell with a high concentration of KH granules at the center of the field. The membranes of both granular and the horny cells are marked thickened (TM). The thick membrane is continuous with the attachment plaque of the now modified desmosome. The interdesmosomal discs (►◄) appear to detach from the attachment plaques and form part of the intercellular plate (ICP). 17-week vitamin a deficiency. Vestopal, UA and LC.

X 67,200


HC
GC
HC
ICP
KH
TM
ICP
32

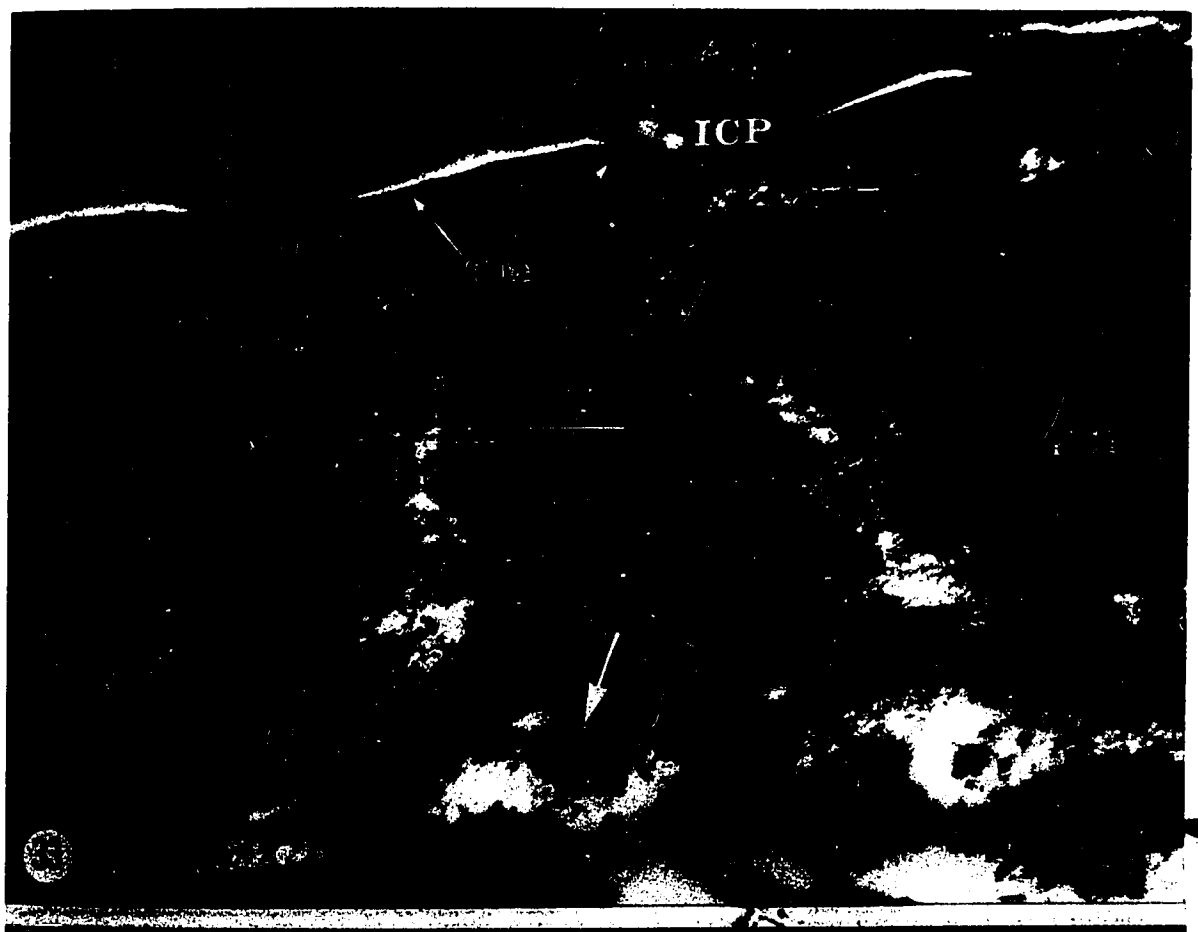

HC
GC
KH

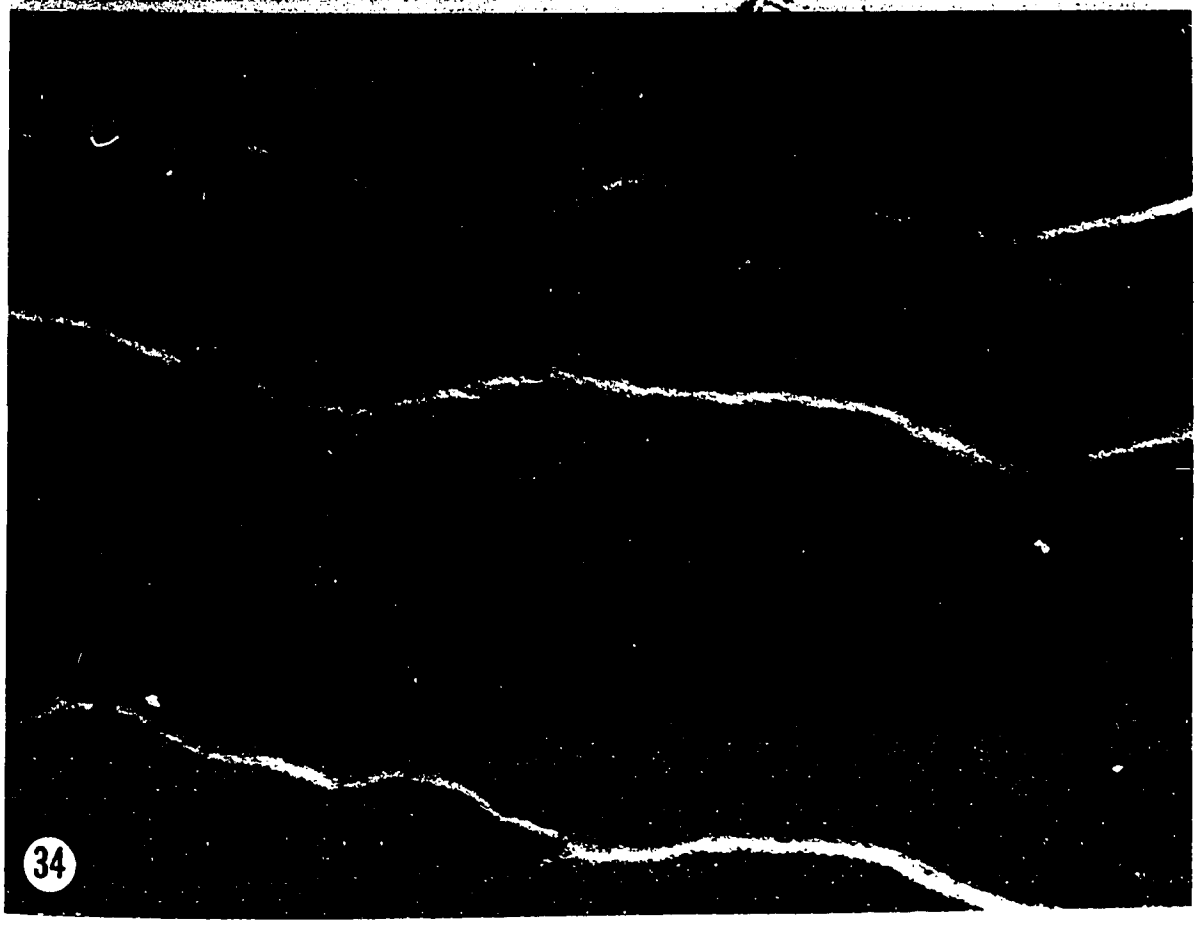
Figure 33. A picture showing a granular cell immediately adjacent to the horny layer. The thickened plasma membrane (TM) can easily be identified. A discharged membrane-coating granule in the intercellular space is indicated by an arrow. 17-week vitamin A deficiency. Vestopal, UA and LC.

X 87,500

Figure 34. High magnification of stratum corneum showing the fibrillary appearance of these horny cells. 17-week vitamin A deficiency. Vestopal, UA and LC.

X 87,500


ICP


34


TM
KH
33

The intercellular discs of desmosomes were not only "detached" from the apparently modified attachment plate, but also extended into intercellular spaces between the desmosomes together with the MCG and other materials to form intercellular "plates" of horny cells (Figs. 32, 33, 34).

3. Langerhans Cells

Langerhans cells were not found in any specimens of the normal trachea or urinary bladder. Numerous studies by other workers, likewise, have made no mention of Langerhans cells in either of these tissues (Rhodin and Dalhamn, 1956; Rhodin, 1966; Leeson, 1962; Hicks, 1965; Monis and Zambrano, 1962a).

Langerhans cells were, however, observed in the epidermoid epithelium of both tissues. Their morphology and their relationship to the epidermoid epithelium were indistinguishable in the two tissues, so that the following description applies to both of them. A total of approximately 200 Langerhans cells was studied in these tissues.

As in the epidermis (Zelickson, 1967), the Langerhans cells were more often located in the basal or suprabasal region than in the more superficial levels of the epithelium. They appeared either singly (Figs. 35, 36) or in groups of two or three cells between the keratinocytes (Fig. 36). Langerhans cells were also observed in the lamina propria, just under the basement membrane (Figs. 37, 38).

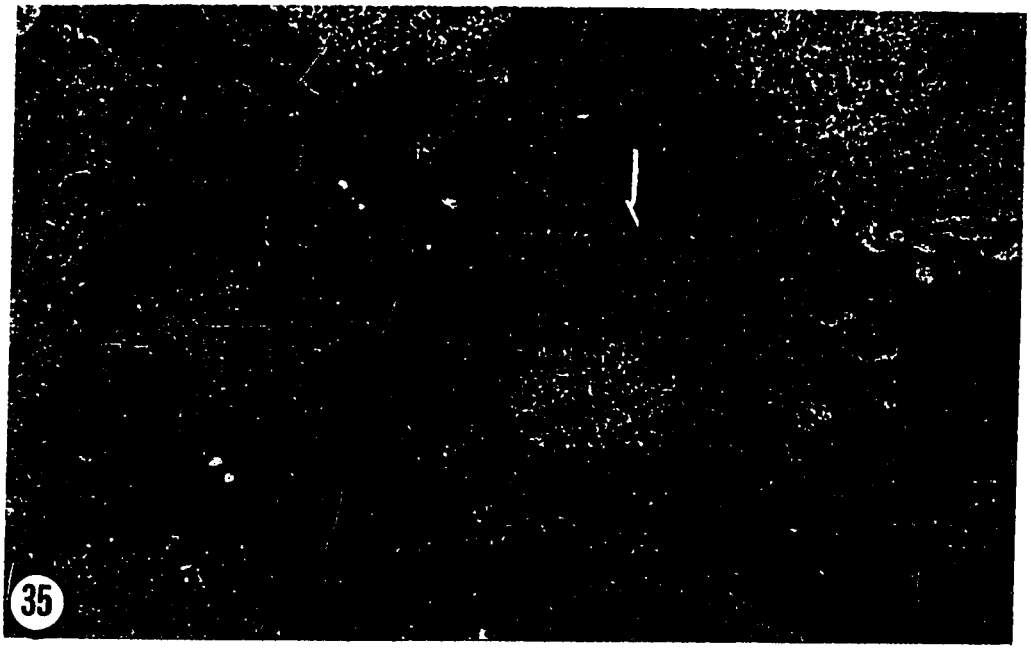
The cell shape was highly irregular, as judged by the many slender processes. The plasma membrane was of much higher density than that of the adjacent keratinocytes (Figs. 35, 36, 41). It was also deeply infolded at points around the periphery (Figs. 36, 40). The nucleus was also irregular and the indented character of the nuclear membrane made it easy to distinguish these cells from the keratinocytes.

Figure 35. Survey micrograph of metaplastic (cornified) urinary bladder epithelium of the rat in vitamin A deficiency. The Langerhans cell is identified by its highly indented nucleus and infolded plasma membrane. The specific granules are not visible at this magnification. A cytoplasmic process of the Langerhans cell is indicated by the arrow.

X 4200

Figure 36. Langerhans cell of metaplastic urinary bladder epithelium. In addition to the features seen in Fig. 35, the cell shows the characteristic well-developed Golgi complex, associated with which are a centriole (c) and the rod-shaped typical Langerhans cell granules (arrow). The plasma membrane has a higher density than that of the surrounding keratinocytes.

X 16,500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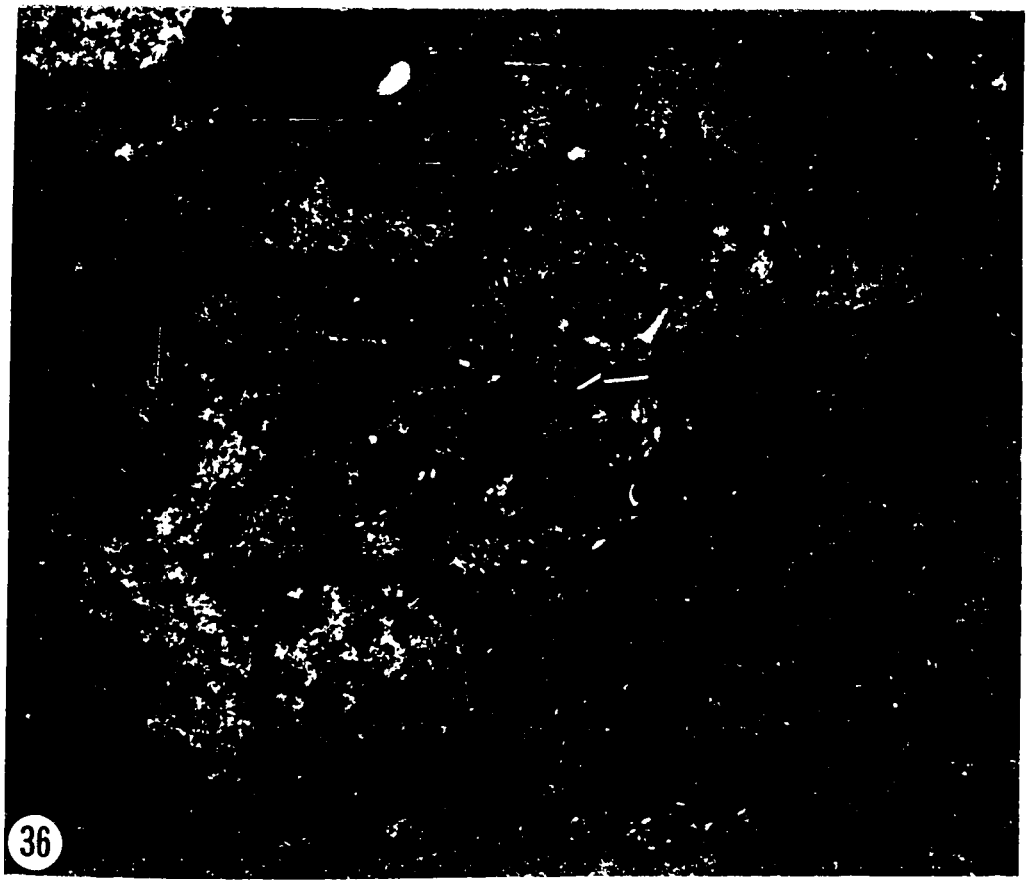

36


35
C

Figure 37. A Langerhans cell lying in the lamina propria of the urinary bladder in vitamin A deficiency. The basement membrane of the cornified epithelium follows a winding course (arrows). The cell is surrounded by collagen (c) fibrils and portions of fibroblasts.

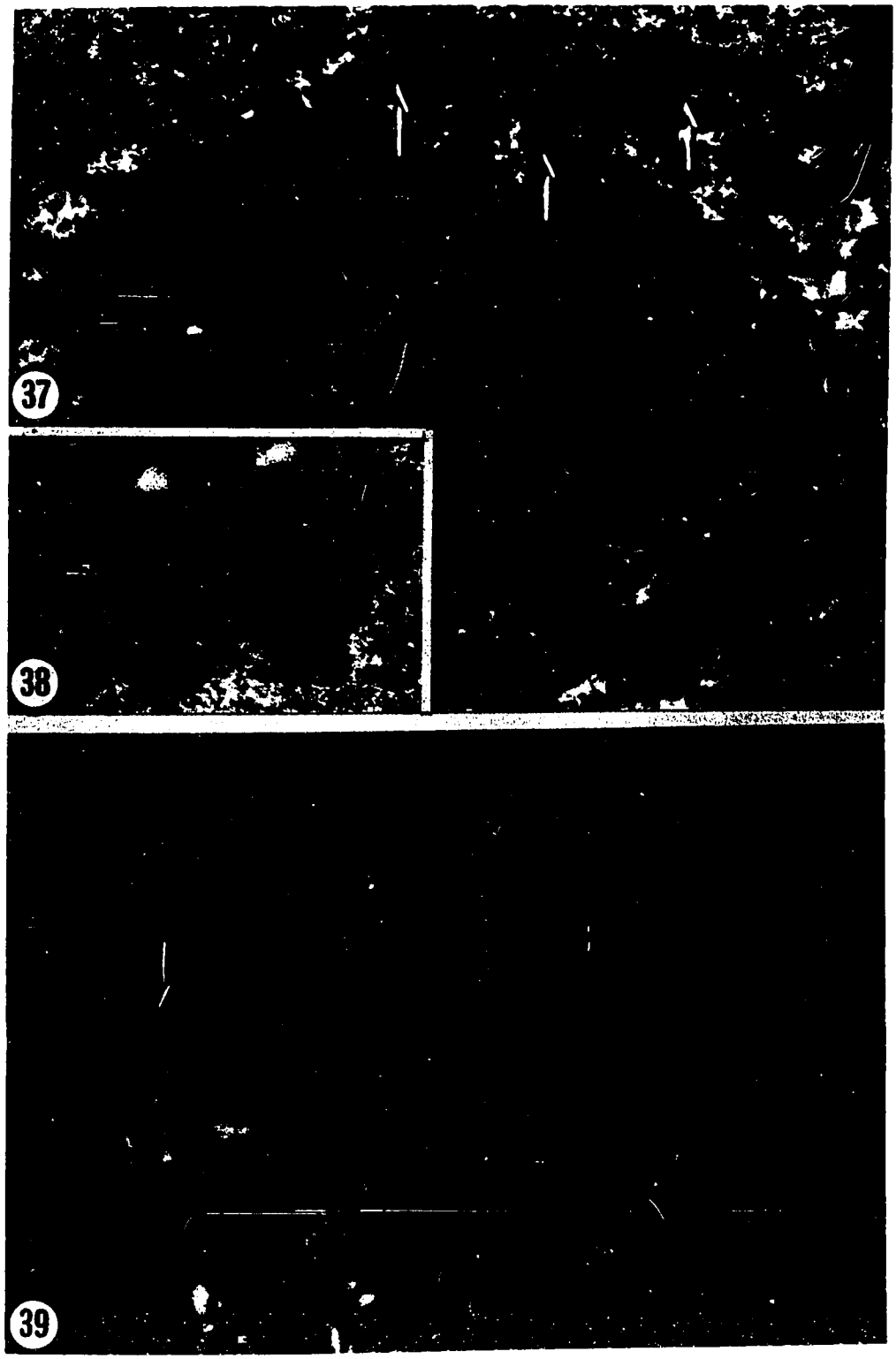
X 12,000

Figure 38. That the cell illustrated in Figure 37 is, in fact, a Langerhans cell is seen by the characteristic granules in this magnified portion of Figure 37.

X 56,000

Figure 39. Metaplastic (cornified) tracheal epithelium showing a portion of a Langerhans cell with a rod-like granule (arrow) and many dense granules. There is also a network of filaments (t).

X 39,900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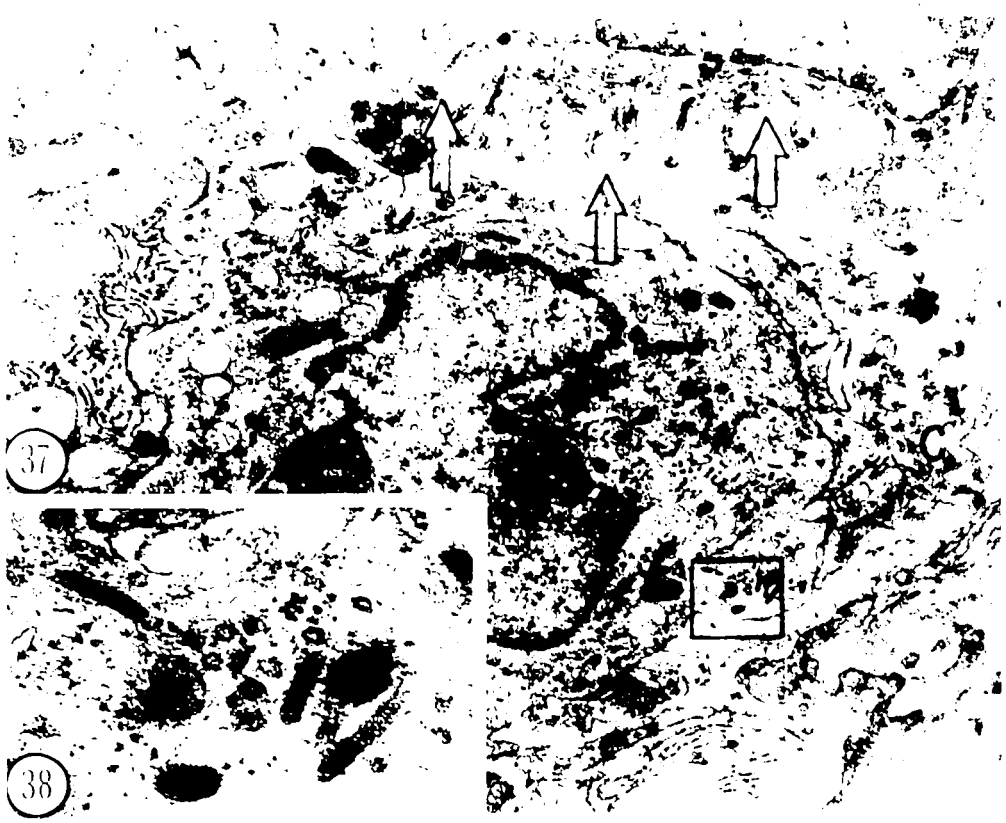

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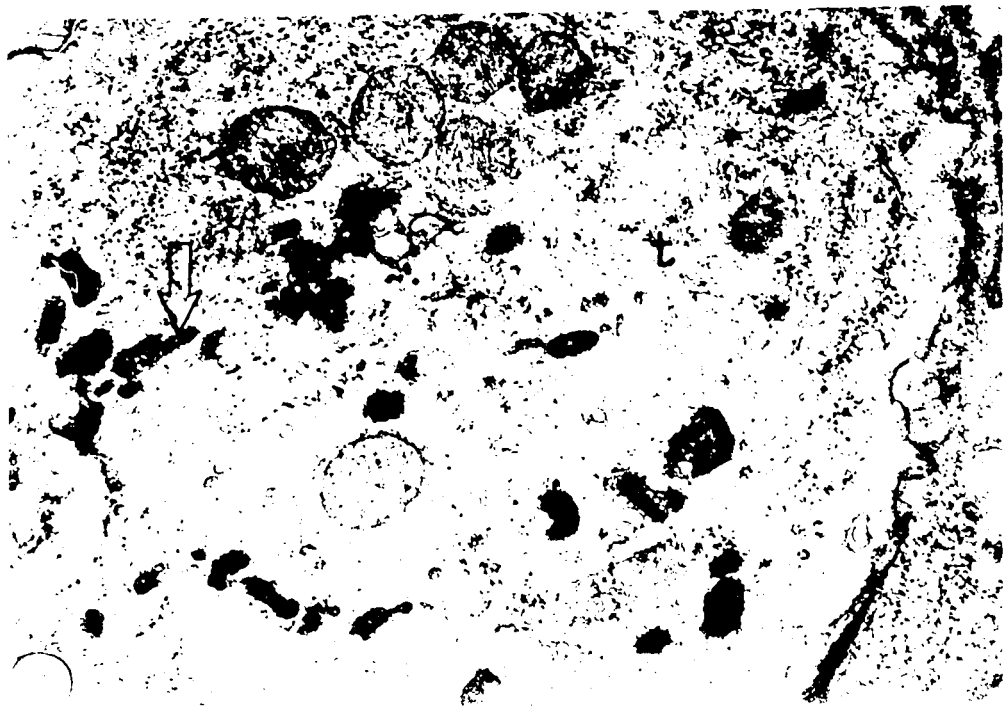


at low magnifications (Figs. 35, 36, 37). The cytoplasm was relatively clear, by comparison with the keratinocytes, although some filaments could be seen at high magnification, especially in the processes. The rough endoplasmic reticulum was not prominent but the Golgi complex was well developed (Figs. 36, 37, 40) and was often seen to enclose the centrioles (Figs. 36, 40). In some cells long microtubules were observed in the cytoplasm, in one case radiating out from the vicinity of the centrioles (Fig. 40).

The characteristic granules of the Langerhans cell, although somewhat less numerous than in published pictures of epidermal Langerhans cells, usually appeared in micrographs as linear structures, each with rounded ends and a striated lamella in the axis of the granule (Figs. 36, 37, 38, 41, 42, 43). The "racket" shape granule, described by others, was sometimes seen (Fig. 42). Occasionally granules with a thin "tail" were observed (Fig. 43).

Granules of a different kind, earlier described by Zelickson (1966), were also found in these cells. These circular or elongated granules with a homogeneous, dense matrix were bounded by a distinct membrane (Figs. 39, 42, 43, 44, 45).

There appeared to exist stages of transition between the dense granules and the characteristic Langerhans cell granules (Figs. 43, 44, 45). Occasionally a single granule appeared to have the striated lamella towards one end and the dense matrix at the other (Fig. 45-d). Some dense granules showed a matrix which, although not striated, lay in an axial positon (Figs. 43-c, 44-b).

Figure 40. From the vicinity of a pair of centrioles (c), lying beside the prominent Golgi appartus, numerous microtubles (arrows) radiate. The image suggests an immediate pre- or post-mitotic phase of the cell.

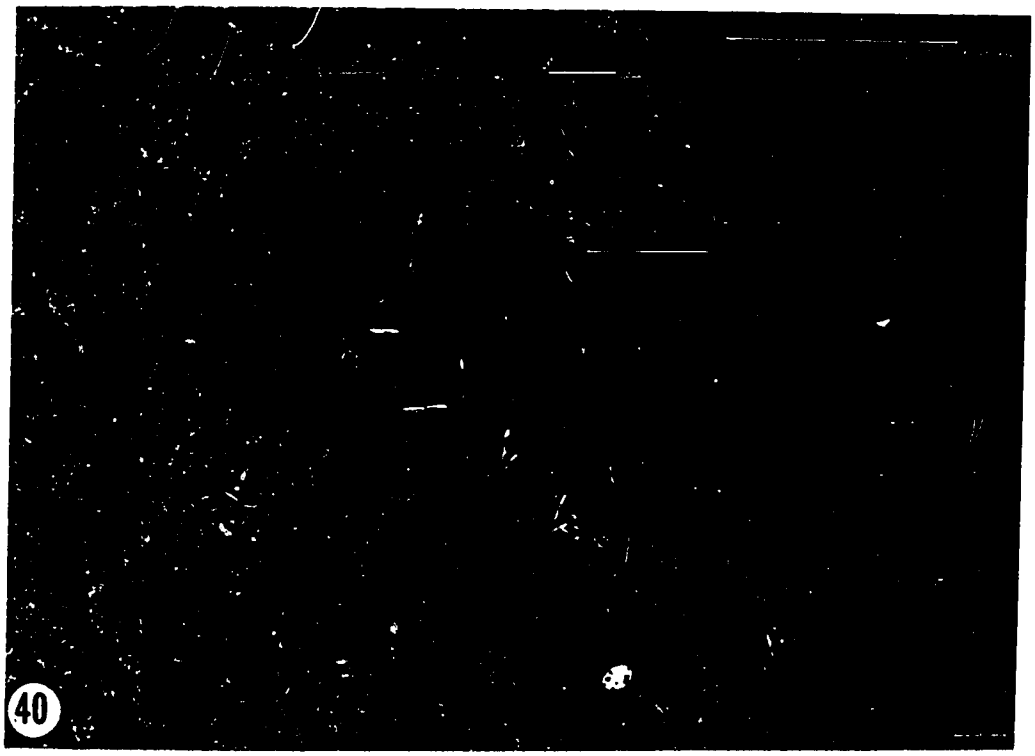
X 20,000

Figure 41. A rod-shaped Langerhans cell granule having a striated axial lamella. A racket-shaped profile is also shown.

X 120,000

Figure 42. Two racket-shaped granules and several dense granules.

X 64,000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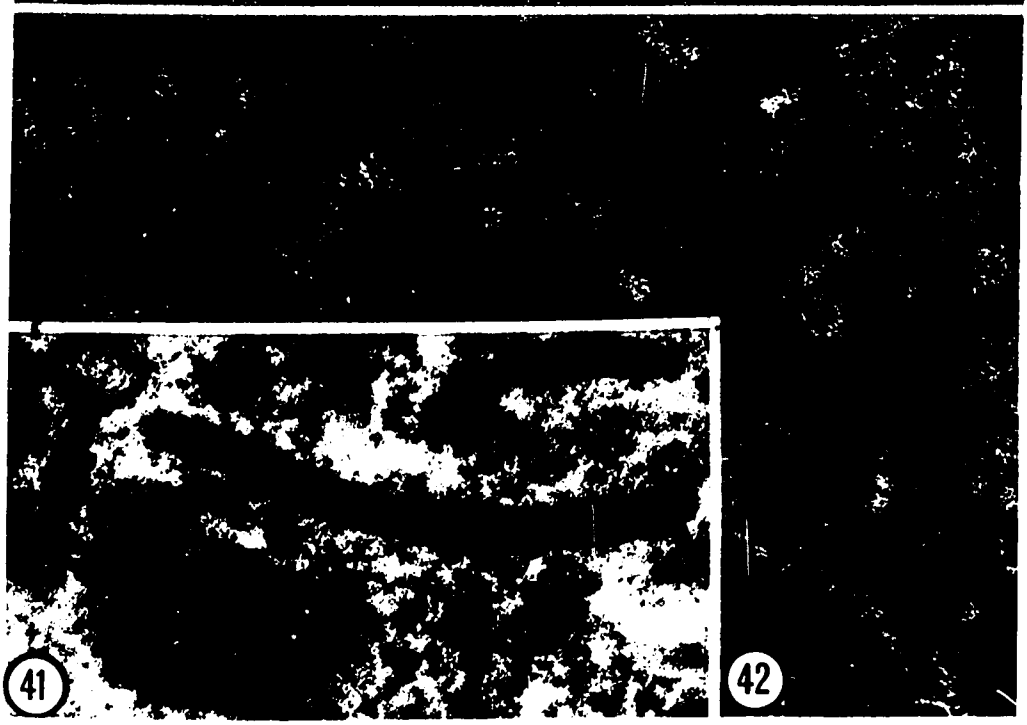
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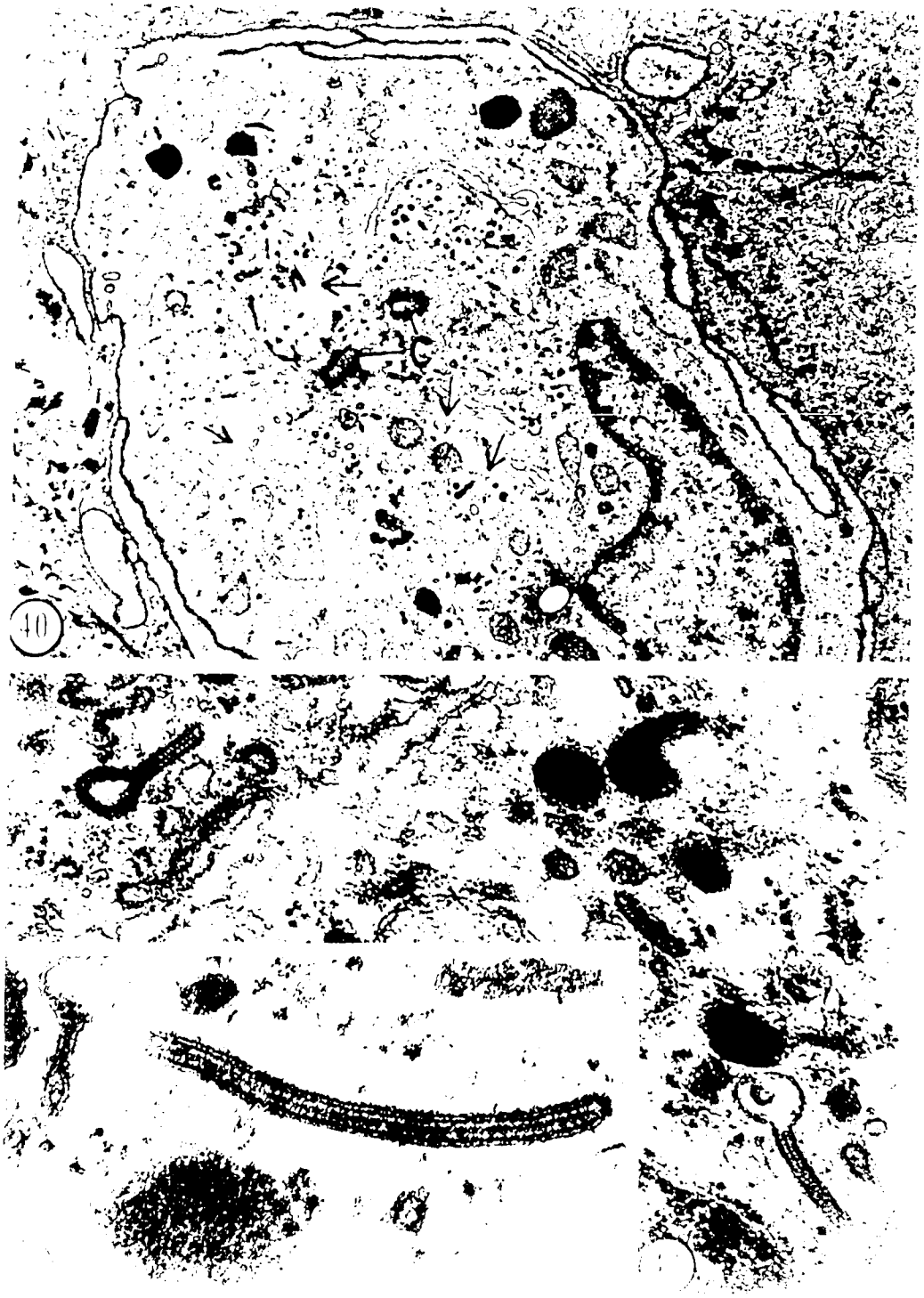
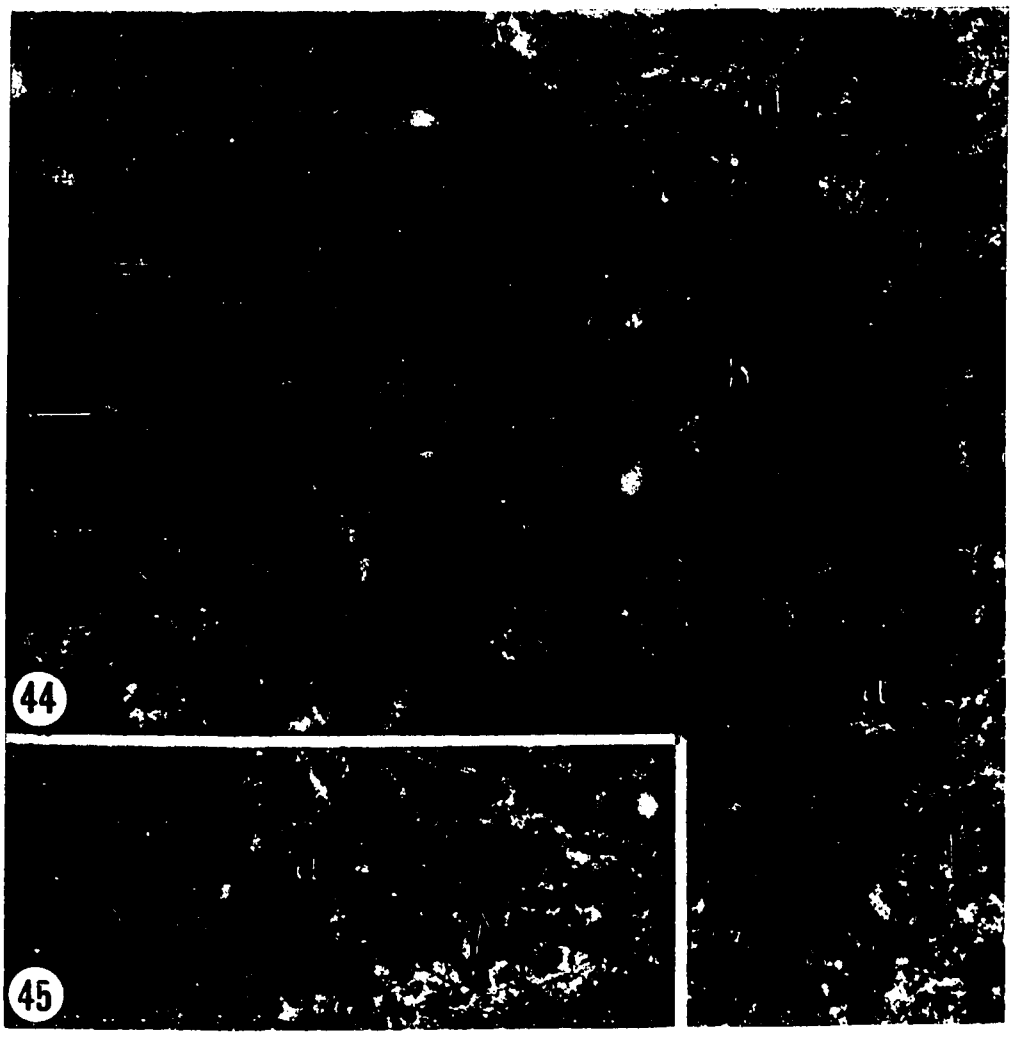

40

Figures 43, 44, and 45. Stages suggesting a transition between rod shaped and dense granules (or vice versa). (a) typical dense granule, (b) elongated dense granule, often showing incomplete filling of the membranous envelope with the dense contents, (c) very attenuated granule, (d) granule with striated axial core and dense terminal swelling, (e) "typical" Langerhans cell granule with striated axial core. Figure 43 shows, in addition, a type of granule (arrows) having a thin tail extending from the rod.
Fig.43 X 64,000, Fig. 44 X 48,000, Fig. 45 X 46,000


43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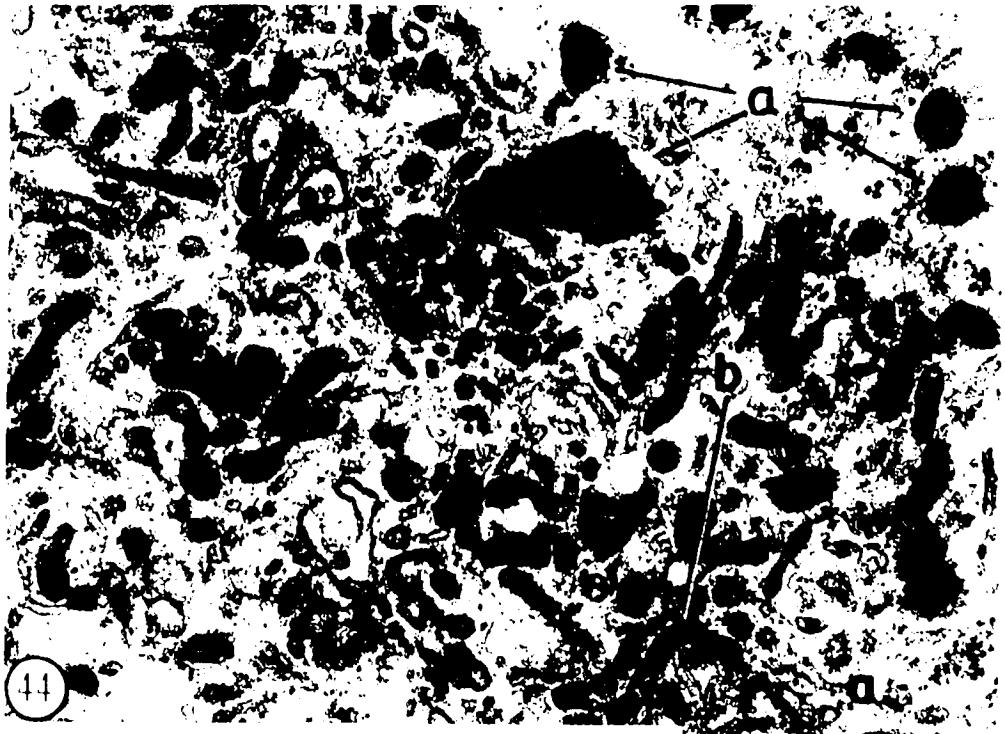
45

43



44

45

4. Chick Embryo Skin Cultured in Chemically Defined Medium (Waymouth MB 752/1)

(a) Six-day chick embryo skin examined at the time of explantation.

Epidermis of six-day old chick embryo was composed of a single layer of columnar cells (basal layer) and a single layer of superficial flattened cells, known as periderm (Fig. 46).

The basal layer was separated from the underlying dermis by a basement membrane. The basal oells contained a large oval nucleus and two or three nucleoli. The RER was sparse. However, free ribosomes and polysomes were prominent in the cytoplasm. Elongated and sometimes branched mitochondria were seen scattered through the cytoplasm. In many cases, dense mitochondrial granules were observed. The GA was supranuclear in position. A few invaginations of cytoplasmic membrane adjacent to the basement membrane were coated with fuzzy material (Fig. 47). Vesicles of similar coating material were also observed in the basal cytoplasm. A few desmosomes and occasional tight junctions were observed between basal cells (Fig. 46). Some collagen fibrils were observed beneath the basement membrane (Figs. 46, 47).

The peridermal cells consisted of a single layer of flattened cells (Fig. 46) in which the ribosomes and mitochondria were scattered throughout the cytoplasm. The RER was again sparse. The membranes of adjacent peridermal cells were highly convoluted. At their superficial margin adjacent peridermal cells formed junctional complexes. A few desmosomes were seen between periderm and basal cells. A few short microvilli were present on the free surface.

(b) Six-day skin, one day in vitro.

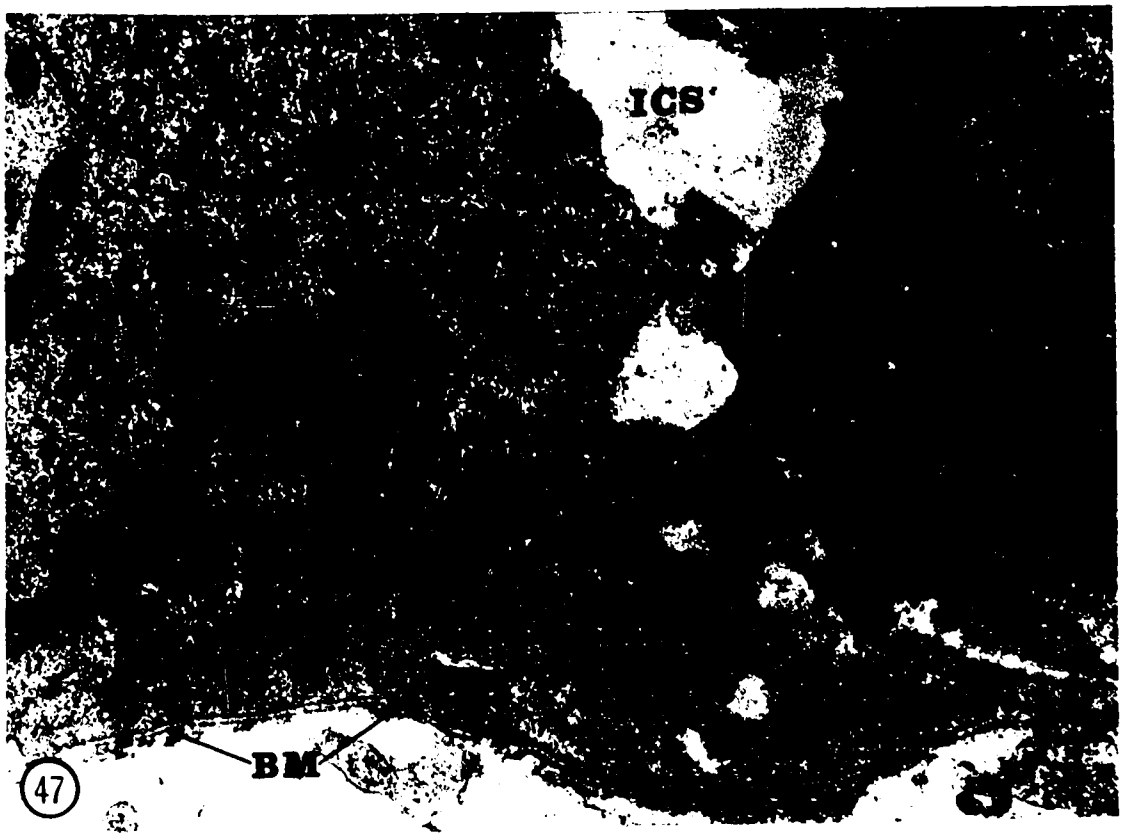
Figure 46. Six-day old chick embryo skin at the time of explantation. The epidermis is composed of columnar basal cells or "epidermis proper" (E) and flattened-peridermal (P) cells. A thin basement membrane (BM) separates the dermis (D) from the epidermis. Epon, UA and LC.

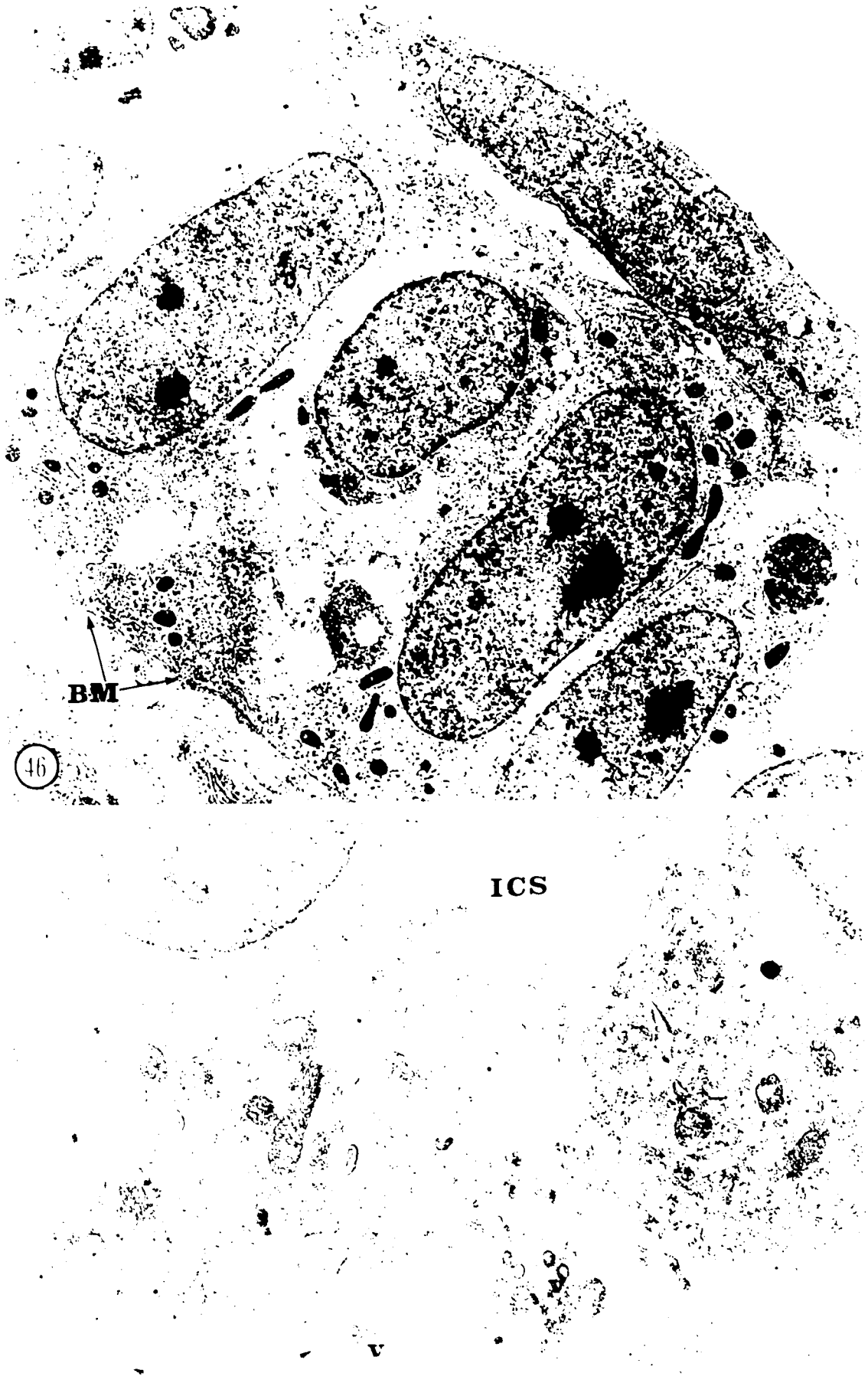
X 7200

Figure 47. Six-day old chick embryo skin at the time of explantation. A basement membrane (BM) is seen beneath the epidermal cells. The RER of the epidermal cells is scanty. A large number of free ribosomes is present in the cytoplasm. Epon, UA and LC.

X 17,000


P
E
D
46


ICS
BM
47


BM
46
ICS
BM

The epidermis was still mainly two-cell layers thick, with only occasional intermediate cells between the basal layer and periderm (Fig. 48).

Desmosomes appeared more prominent and better developed and some filaments were present. Mitoses were observed in the basal layer (Fig. 48).

Microvilli of some peridermal cells were flattened and some glycogen granules were observed in the cytoplasm. Others remained the same as in the six-day old control skin.

(c) Six-day skin, two days in vitro.

The epidermis was four-cell layers thick. The structure was quite similar to that of the previous stage, except for the following differences. The desmosomes appeared more numerous and hemi-desmosomes were observed between basal and peridermal cells. Fine filaments (keratin), sometimes sparsely distributed, at other times in small bundles, were now observed in the epidermal cells. The RER appeared more developed; distended cisternae were sometimes observed in the basal cells; the GA was prominent.

The periderm remained single layered. More glycogen granules accumulated in the periderm.

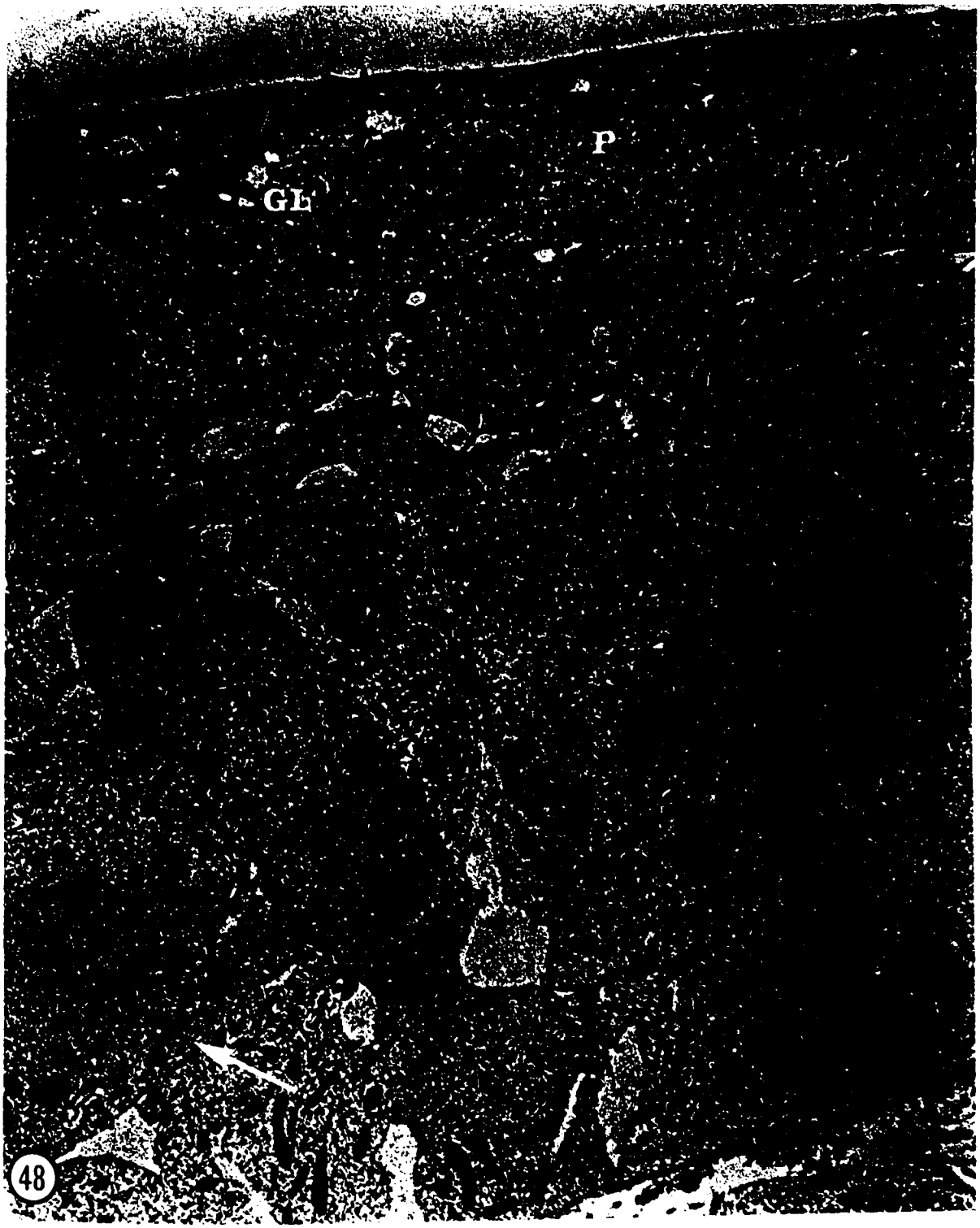
(d) Six-day skin, four days in vitro

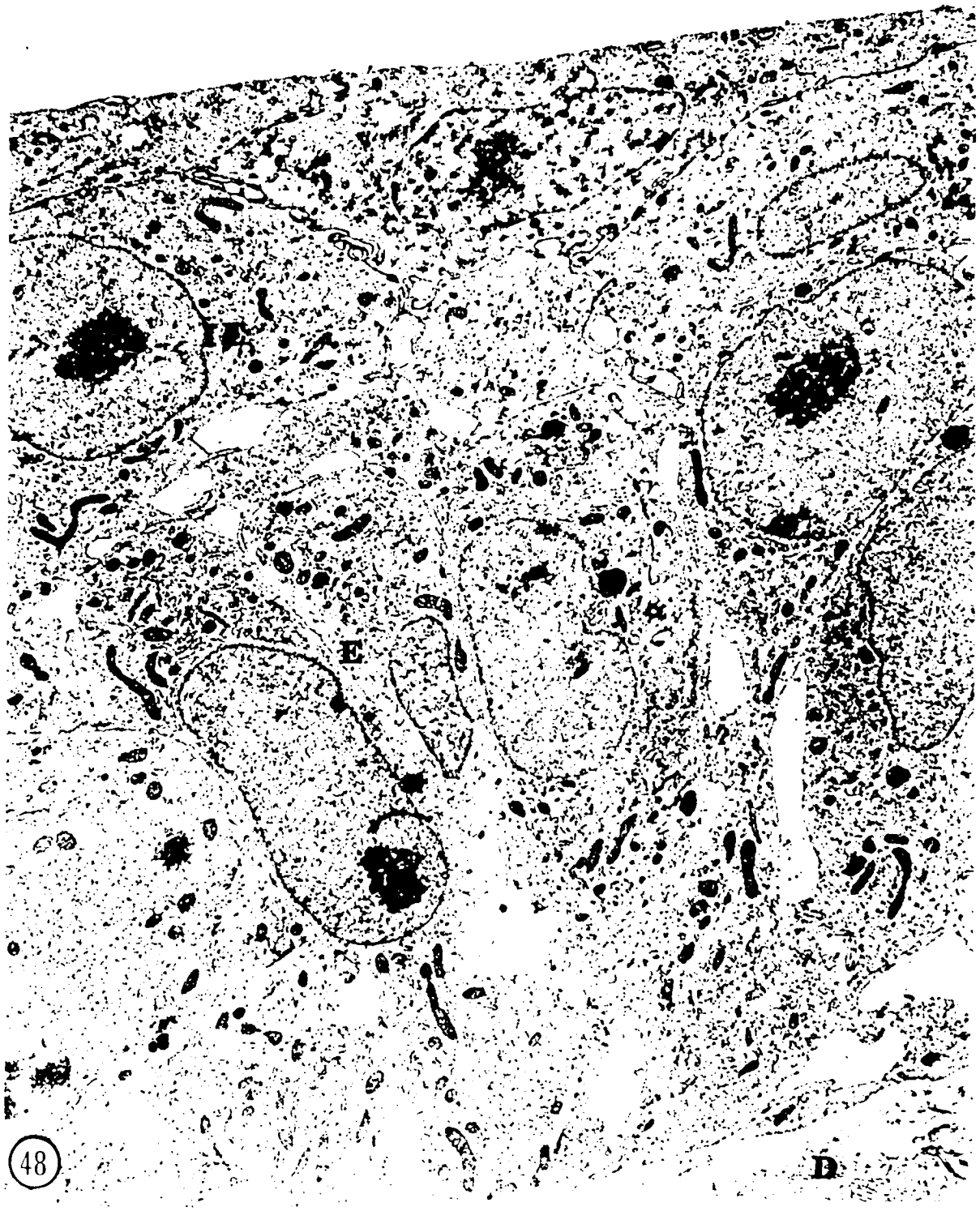
The skin was five or six layers in thickness, of which usually three layers belonged to the "epidermis proper" and two or three layers to peridermal cells (Fig. 49).

In the epidermis the basal cells remained columnar or cuboidal in shape. RER was prominent, and cisternae were distended with finely granular electron opaque material (Fig. 50). The GA was sometimes located in a hypo-nuclear position. The mitochondria appeared more

Figure 48. Six-day old chick embryo skin, one day *in vitro*. The epidermis (E) proper remains single layered except for occasional presence of intermediate cells (IC). The periderm (P) is one layer in thickness, and cell membranes are highly convoluted. A moderate amount of glycogen (GL) is present in peridermal cells. A mitotic cell is indicated by an arrow. Vestopal, UA and LC.

X 5700

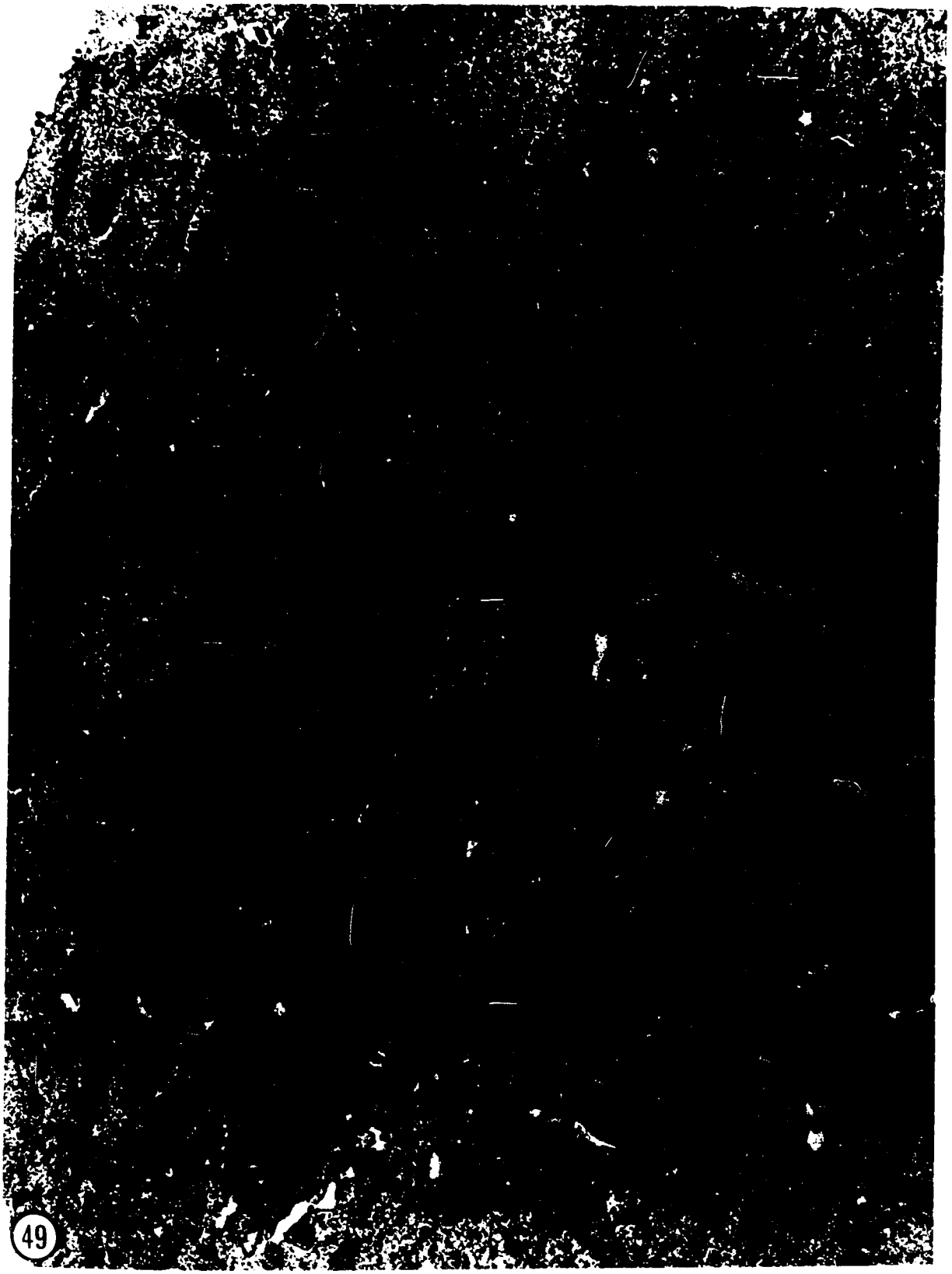

P
GL
48



48

Figure 49. Six-day old chick embryo skin, four days *in vitro*. In the epidermis, the basal cells (BC) are columnar in shape and have well developed desmosomes. Keratin fibrils can be seen in the cytoplasm. Two or three layers of cells distal to the basal cells are squamous in shape and have quite prominent keratin fibrils in the cytoplasm. Peridermal (P) and subperidermal (Sbp) cells can easily be distinguished from the epidermal cells by the presence in their cytoplasm of the peridermal granules (PG) and few keratin fibrils. Epon, UA and LC.

X 9500


49


P
PG
Sbp
E
BC

numerous and mainly located basal to the nucleus. Keratin fibrils were present here-and-there in the cytoplasm (Fig. 50). The two layers of cells distal to the basal cells were either cuboidal or squamous in shape. More than one collection of GA profiles was sometimes found in the basal cells. Multivesicular bodies, dense bodies, and myelin figures were also observed. Occasional cilia were found.

One or two layers of cells beneath the peridermal cells will be referred to as subperidermal cells because they showed some structural differences from surface peridermal cells. The tight junctional complex was still present on the superficial margin of the peridermal cells, but the convolutions of the membranes between cells had largely disappeared.

The most characteristic feature of the subperidermal cells at this stage was the presence of electron dense bodies of an oval, round, or irregular shape and varying in size from few hundreds of Angstroms to several microns (Figs. 49, 51). They were called corpuscula cribriformia by Mottet and Jensen (1968), and peridermal granules by Parakkal and Matoltsy (1968). The latter term will be used in this report. The peridermal granules appeared to consist of interlacing strands of electron dense, structureless cords of about 300-350A° in diameter (Fig. 51). At high magnification these strands were seen to be made up of finely granular material. The incidence and size of these granules in the peridermal and subperidermal cells was quite different, the majority of them being found in subperidermal cells and those which were present in periderm were of much smaller size.

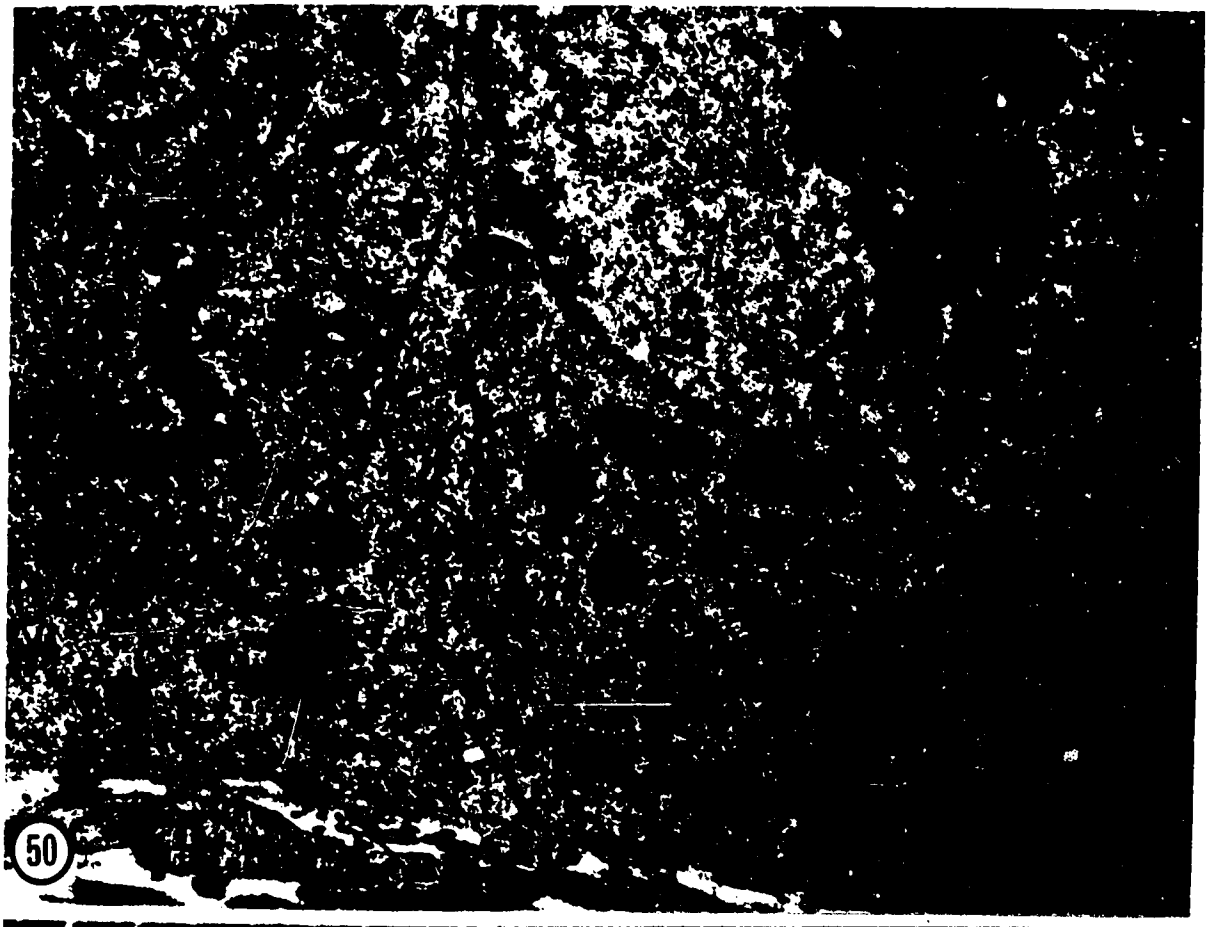
In addition, fibrillary material was more numerous in the subperidermal than the peridermal cells.

Figure 50. Six-day chick embryo skin, four days in vitro. The RER of the basal cells are quite prominent and distended with granular material. A Golgi (G) can be seen at the left upper corner, in a hyponuclear position. The basement membrane (BM) is quite thick and collagen fibres are prominent in the dermis (D). Vestopal, UA and LC.

X 22,440

Figure 51. Peridermal granules from the six-day old chick embryo skin cultured for four days in vitro. The size of these granules ranges from few hundreds of angstroms to several microns. They are made-up by interlacing strands of electron dense cores of about 300-500A° in diameter. Epon, UA and LC.

X 60,000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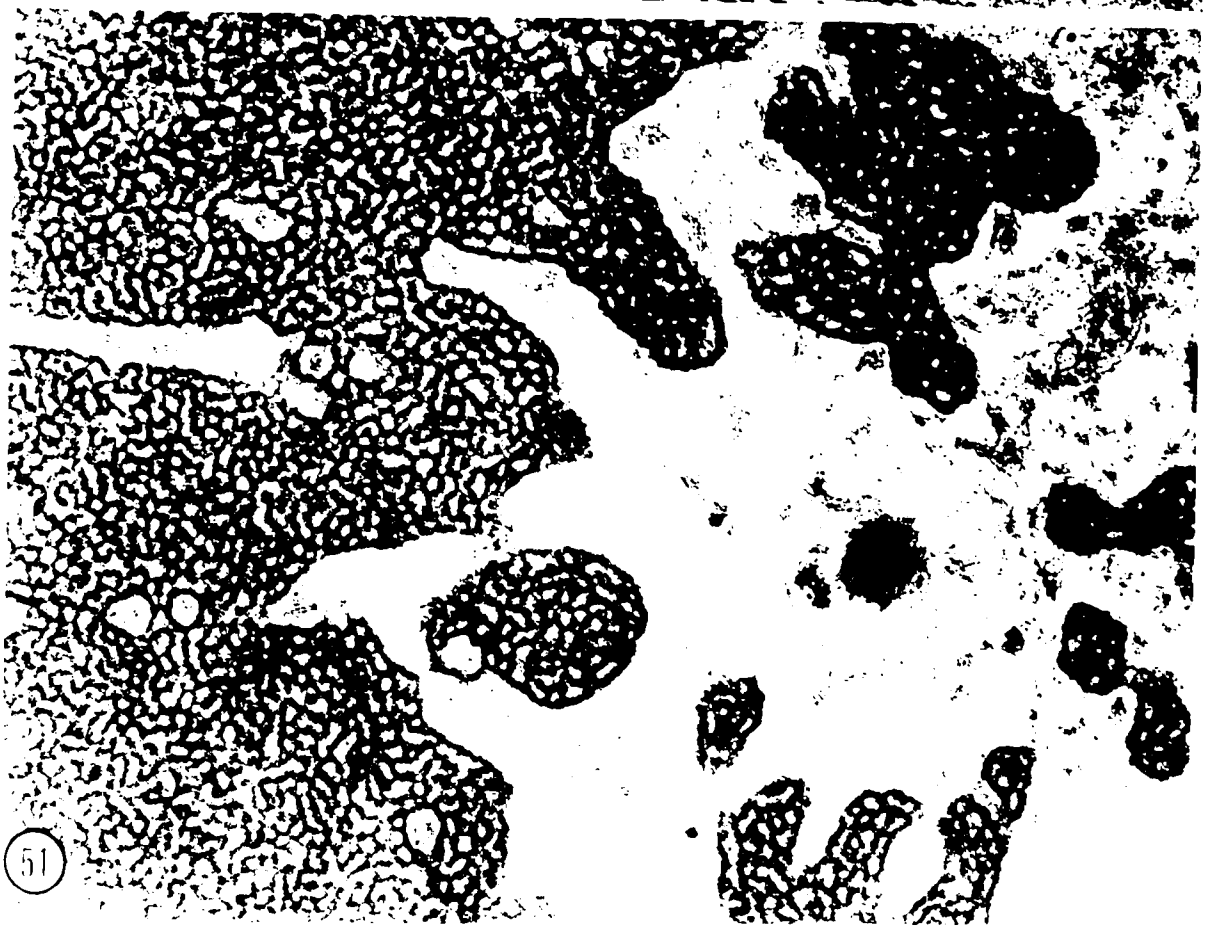
51

50



51

(e) Six-day skin, six days in vitro.

The skin was many layers thick after six days in culture. Both the epidermis proper and the periderm were well devloped. The former consisted of seven or eight layers of cells, while the latter might have as many as 10 or 15 layers.

The basal cells and two layers of cells distal to the basal cells were similar to those of four-day cultures, except that keratin appeared more prominent, and the desmosomes were better developed. These cells are comparable to the basal and spinous cells of mammalian epidermis.

Epidermal cells superficial to these were progressively more flattened toward the subperidermal cells (Fig. 52). Large amounts of keratin fibrils were present in the cytoplasm. In sectioned material the nuclei of many of these cells were not seen. Mitochondria and other organelles, though not prominent, were randomly distributed in the cytoplasm. KH granules (though small) were observed both in the cytoplasm and in the nucleus of the cells next to subperiderm. This stratum is comparable to the granular layer of mammalian epidermis.

The most striking feature of the many-layered subperidermal cells was the presence of a large number of prominent peridermal granules, especially by comparison with the previous stage (Figs. 52, 53). Keratin fibrils were quite prominent in some cells, while few in most of the others. Glycogen aggregates were still present.

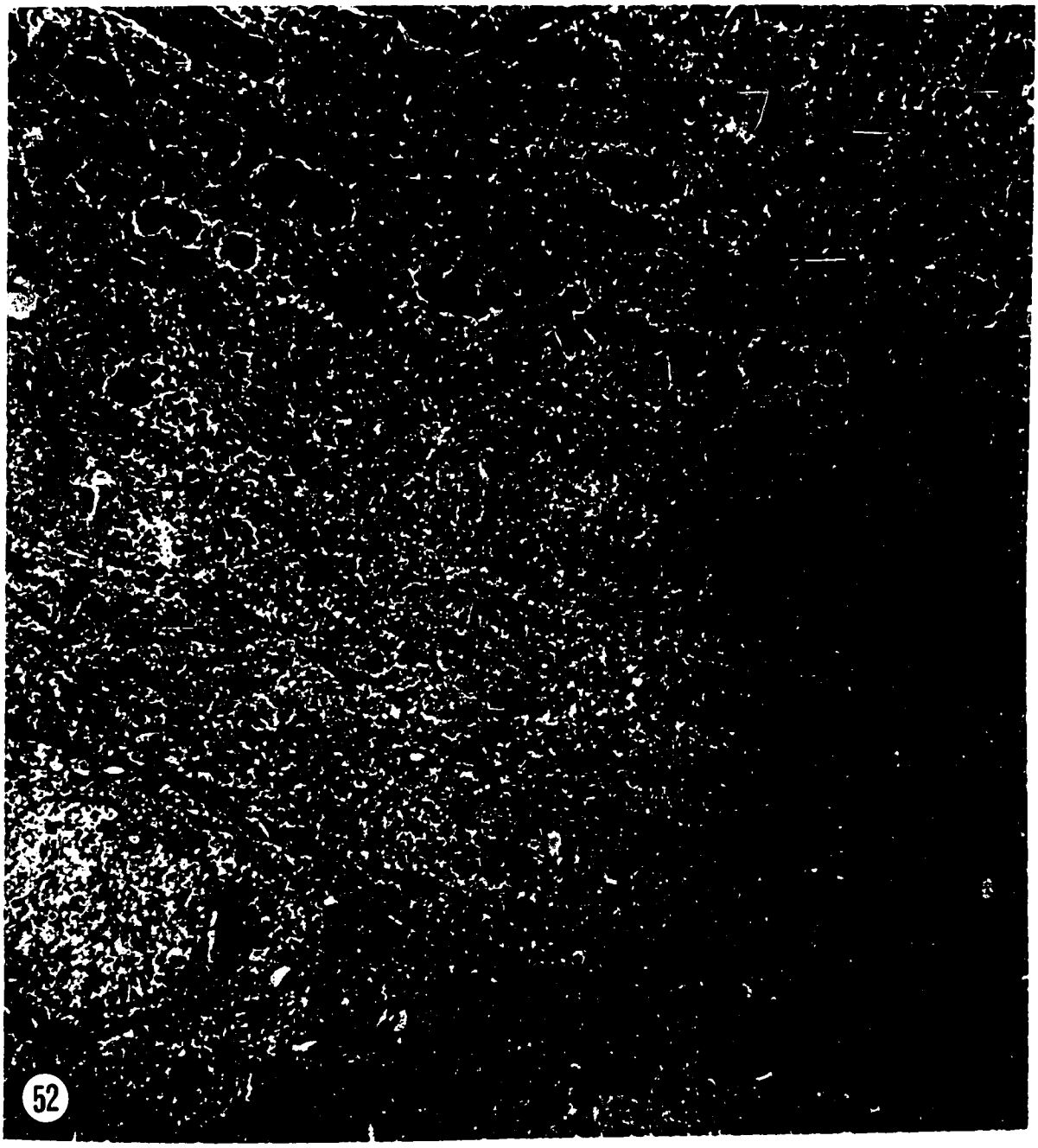
A single layer of peridermal cells lined the free edge of the skin (Fig. 53). Peridermal granules were not observed in most of them.

(f) Six-day skin, eight days in vitro.

The epidermis was well differentiated, and horny cells (stratum corneum) appeared for the first time in this stage (Fig. 54). The basal

Figure 52. Six-day chick embryo skin, six days *in vitro*. Electron micrographs showing part of epidermis and the new, well developed, subperiderm (Sbp). A large amount of keratin fibrils and a moderate amount of glycogen granules (GL) are found in both subperidermal and epidermal cells proximal to the subperidermal layers. A large number of peridermal granules (PG) are present in the subperidermal cells (Sbp). Vestopal, UA and LC.

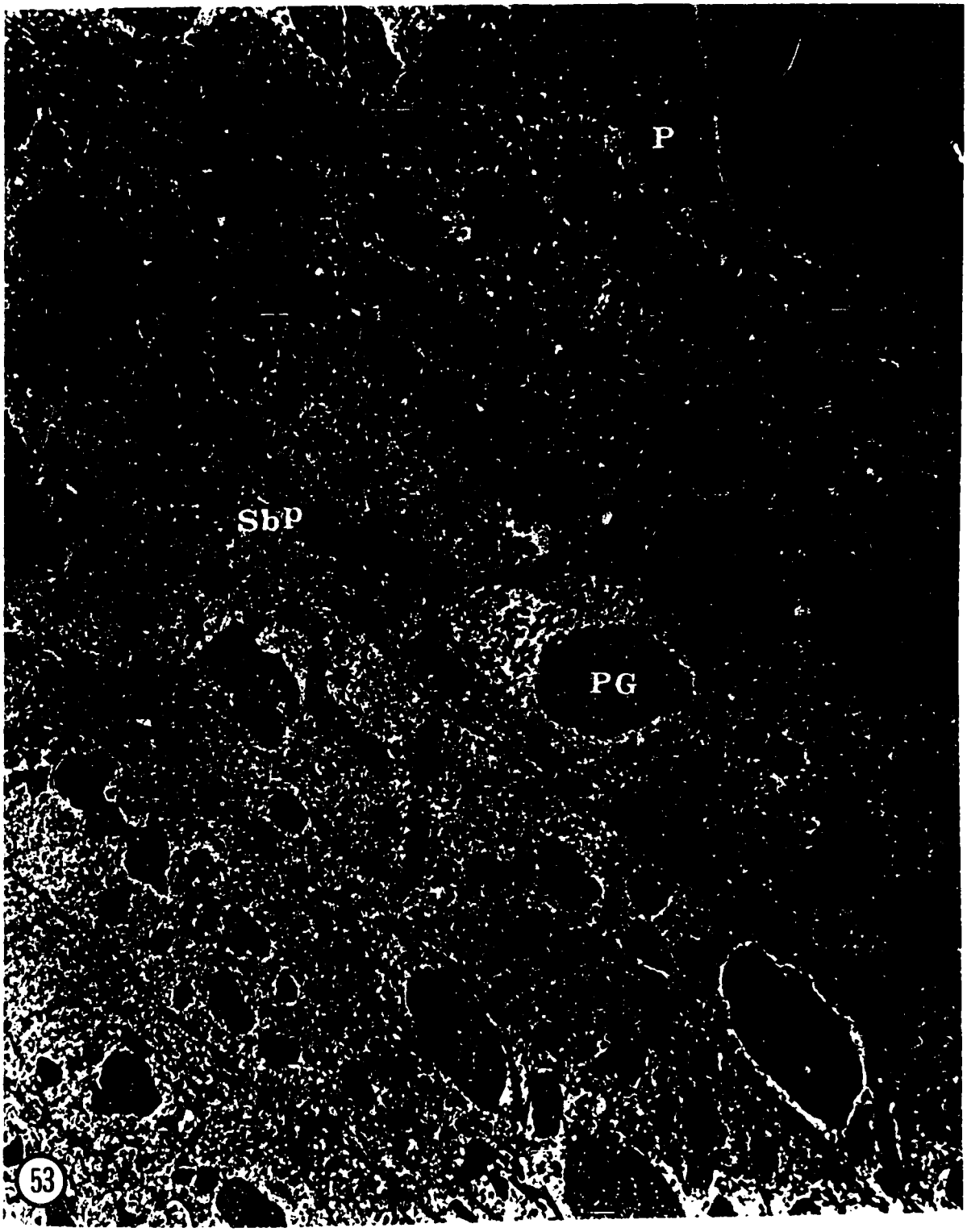
X 5130


52


E
52

Figure 53. Six-day chick embryo skin, six days *in vitro*. The picture shows part of the many layered subperiderm (Sbp) and a single layered periderm (P). Large number of peridermal granules (PG) are shown in the subperidermal cells. Similar granules are found much less frequently in the superficial peridermal cells. In this particular picture, no such granules are seen in periderm. Vestopal, UA and LC.

X 5930


P
SbP
PG
53

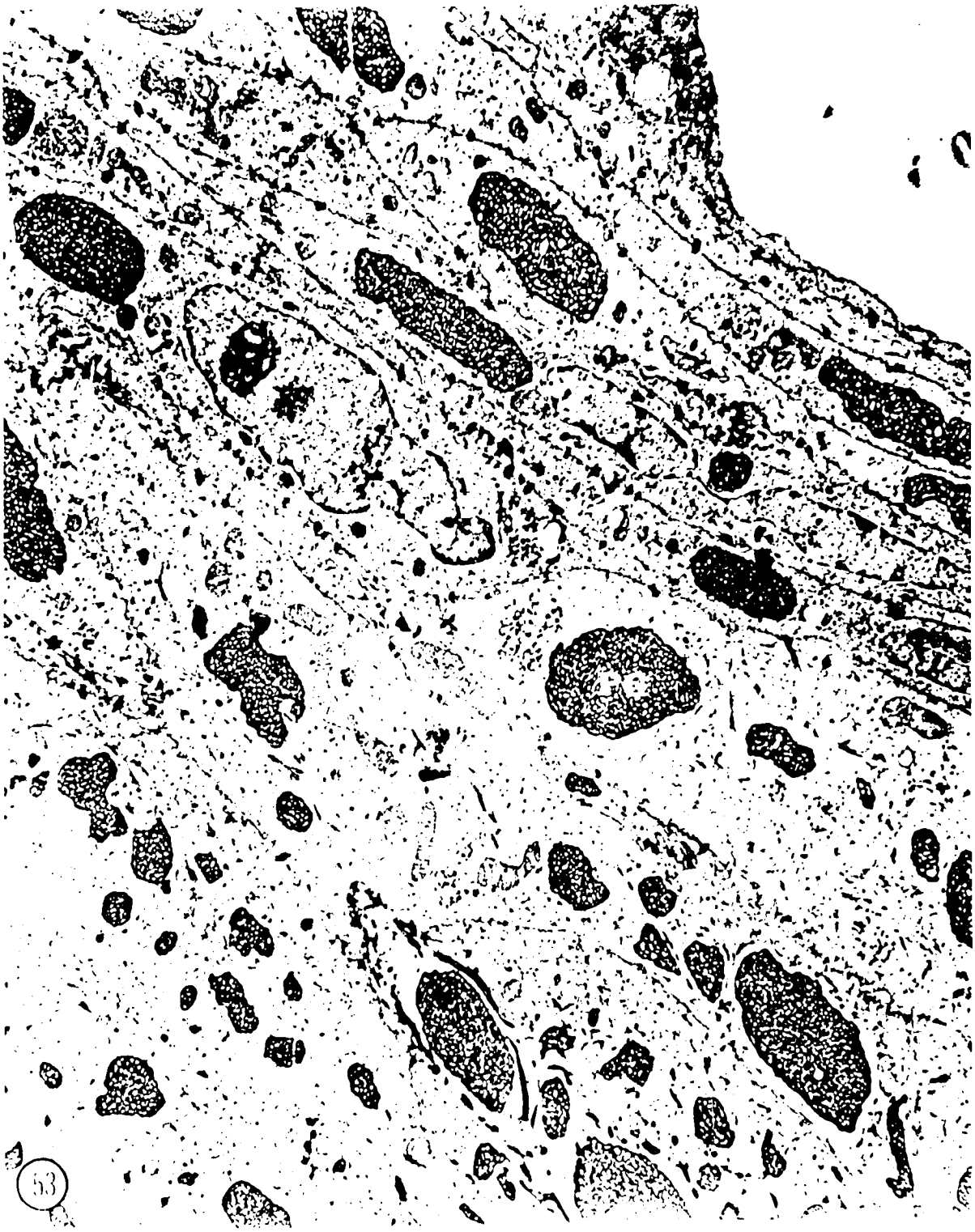
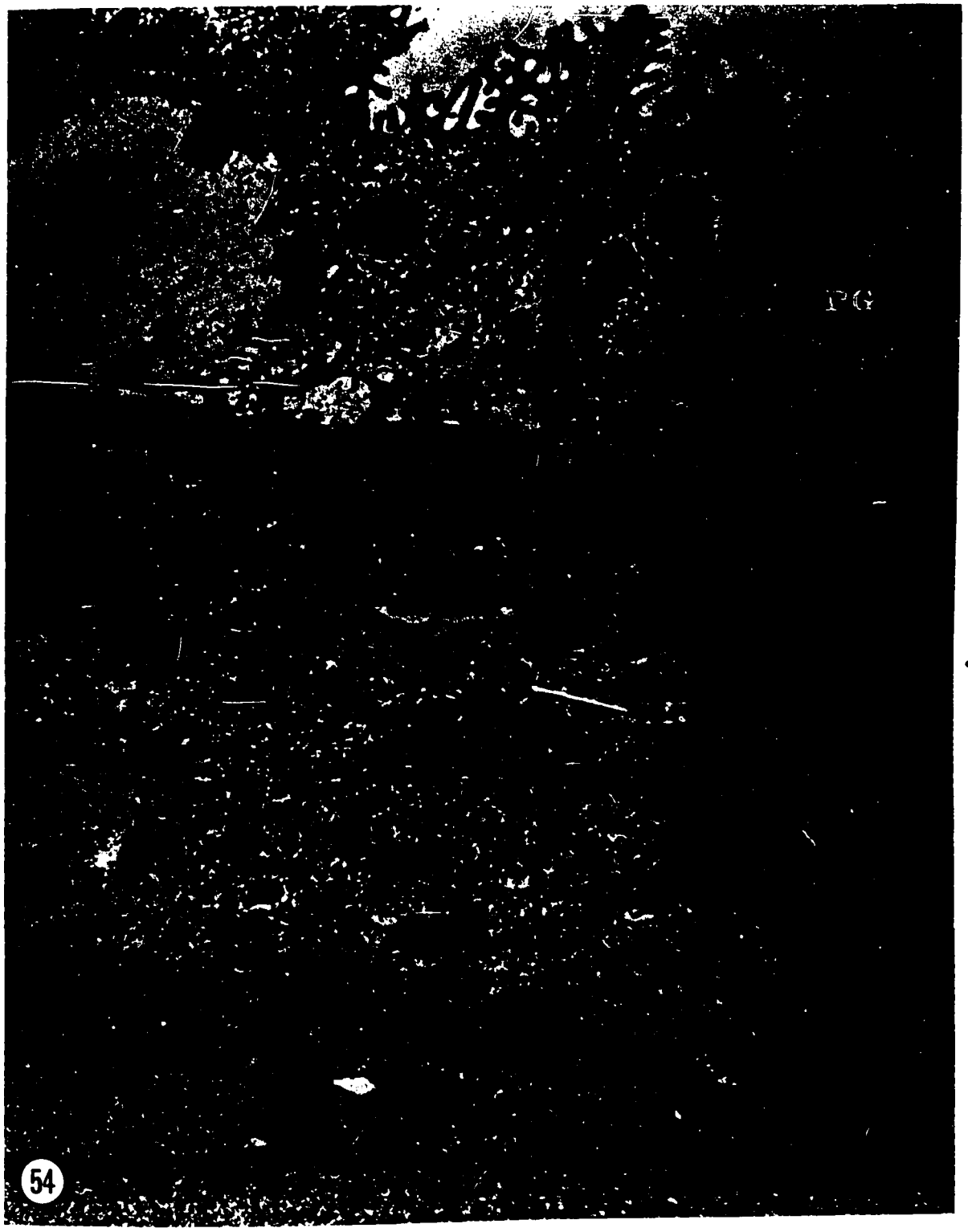

53

Figure 54. Six-day chick embryo skin, eight days *in vitro*. The epidermis is well differentiated, with the appearance of stratum corneum (SC) for the first time. Several layers of granular cells (SG) are present beneath the stratum corneum. Keratohyalin granules (KH) can be identified at this magnification in the granular layer. The keratin fibrils and the KH granules are mainly distributed at the periphery of the cells (arrows). Subperidermal layers (Sbp), which were most developed in six-day specimens, are largely degenerated. However, the single-layered peridermal (P) cells remain intact and show occasional peridermal granules in the cytoplasm. Microvilli are prominent at the free edge of the peridermal cells. Vestopal.

X 13,440


54


Sbp
P
SC
SG
Sb
54

and spinous cells were similar to those of the previous stages. In the spinous and granular layers large amounts of keratin were present (Fig. 55). At a higher level of the epidermis small KH granules accumulated within the cortical part and also in other regions of the cells, including the nucleus (Fig. 56). At higher magnification the KH granules appeared similar to those described for metaplastic tracheal and urinary bladder epithelia. As the keratin fibrils and KH granules increased, other organelles became less prominent. At a still higher level, keratin fibrils and the KH granules coalesced and formed a dense cortical band, bounded by the thickened plasma membrane, measuring approximately 130A° in thickness (Figs. 54, 57). In the central (or axial) area of the cells, between the condensed marginal cytoplasm, lipid droplets and other organelles were seen in a finely granular background. These organelles were believed to undergo dissolution at a later stage.

In the stratum corneum the horny cells (Figs. 54, 57) were of a similar pattern to that described for the late granular changes. However, the cells here were more compact and the central opaque zones were not present or were greatly reduced. The thickness of plasma membrane had further increased (Fig. 57).

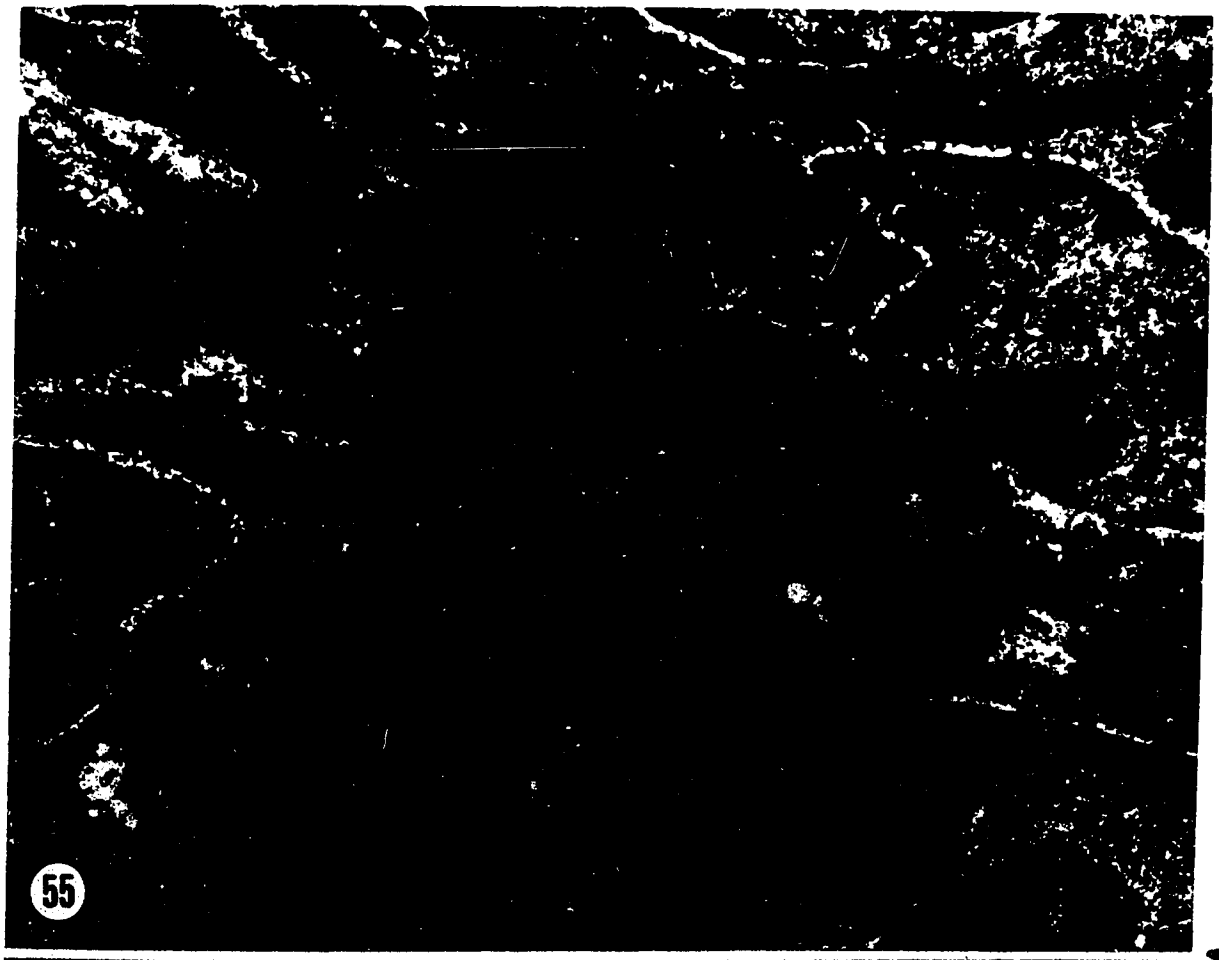
The highly developed subperidermal layers seen on the sixth day of culture had largely degenerated (Fig. 54). One or two layers of electron-light cells present above the horny cells were believed to represent the degenerated subperidermal cells. In places subperidermal cells might still appear normal, and contain peridermal granules (Fig. 54). In most cases the single layered peridermal cells were present. The nucleus was pycnotic and a few peridermal granules could be seen.

Figure 55. Six-day chick embryo skin, eight days *in vitro*, showing portions of prickle cells. Large bundles of keratin fibrils are present in the cytoplasm. In addition, numerous free ribosomes are also observed. Epon.

X 17,600

Figure 56. Six-day chick embryo skin, eight days *in vitro*. Keratohyalin (KH) granules are present both in the nucleus and the cytoplasm of the granular cells. They are small in size and not comparable with those of mammalian skin or of metaplastic epithelia reported in this thesis. Vestopal.

X 36,280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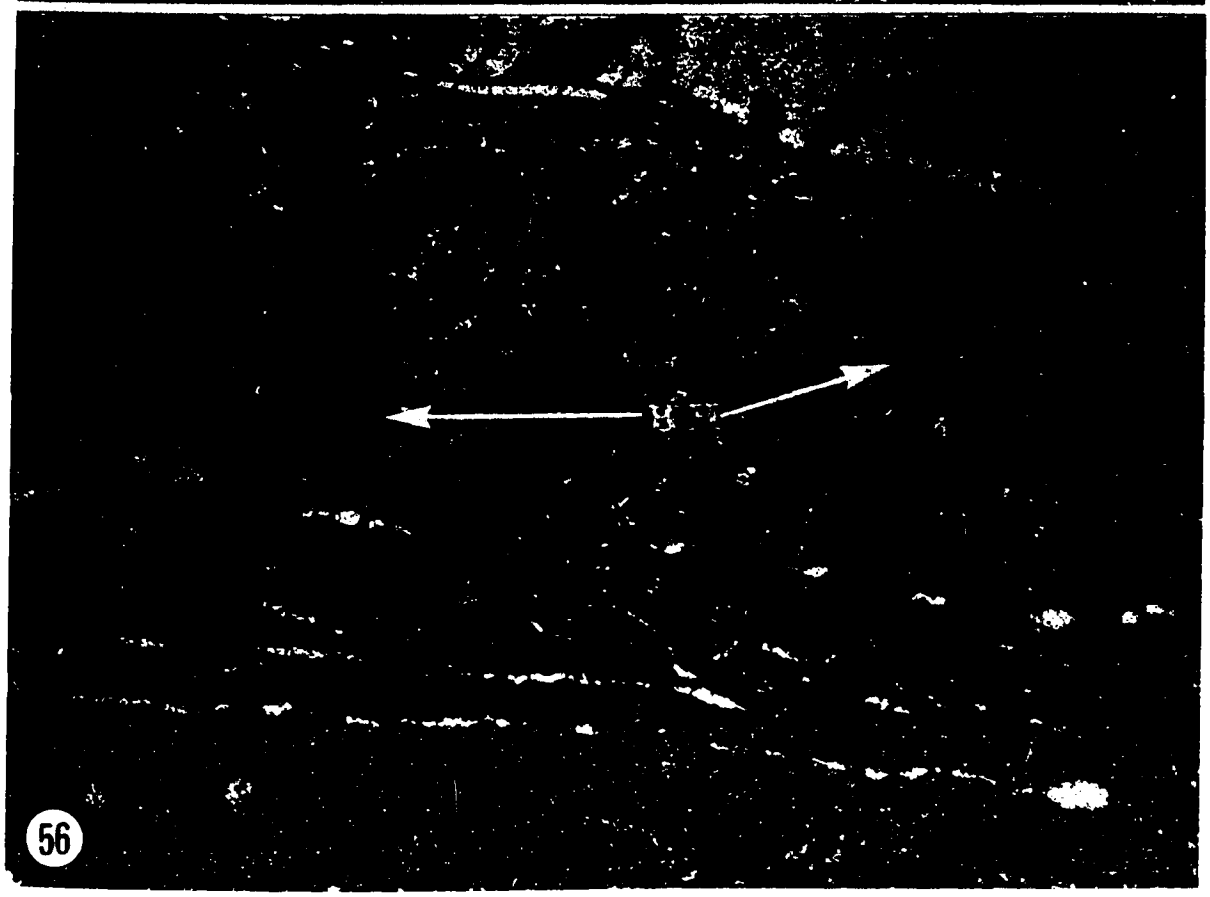
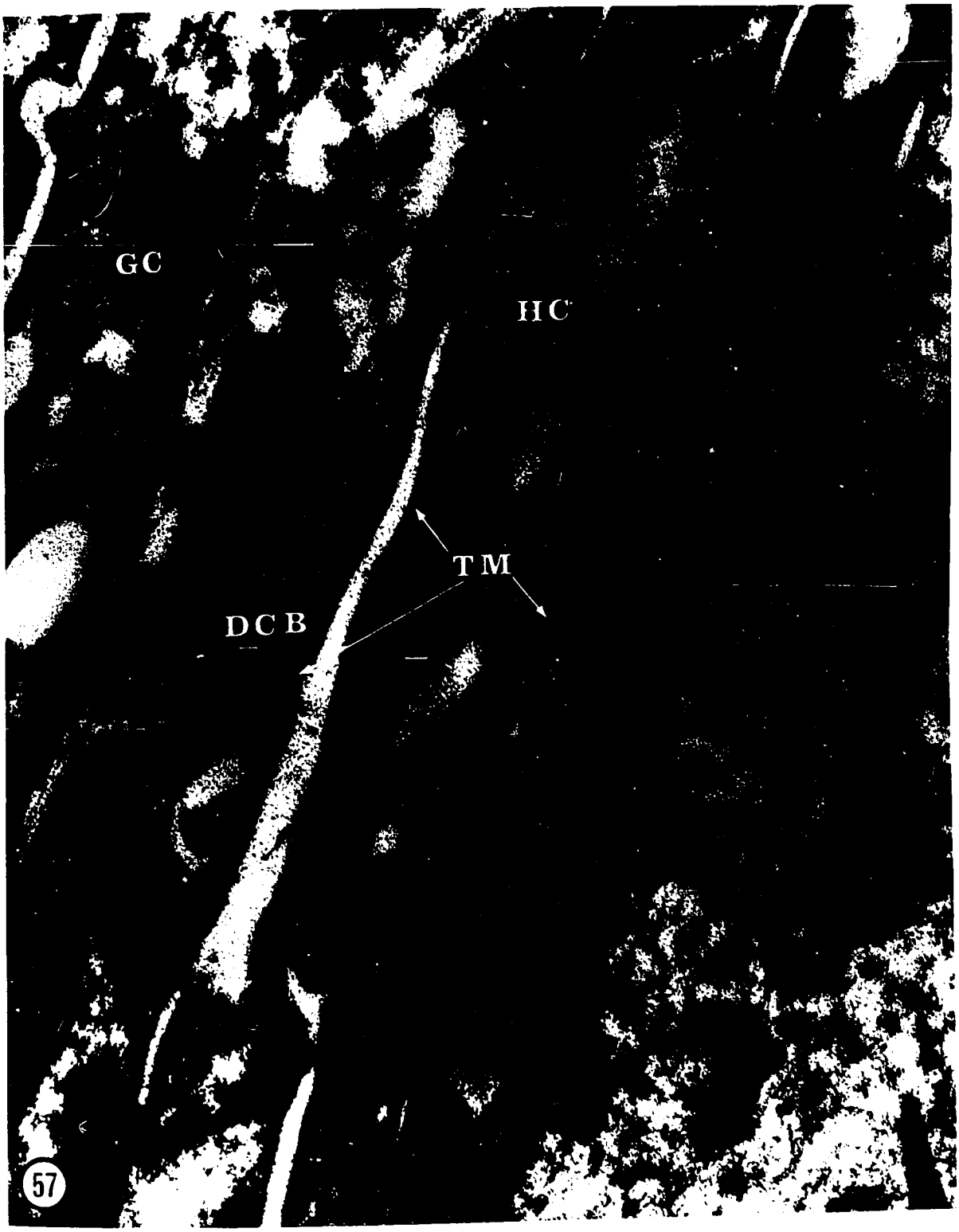
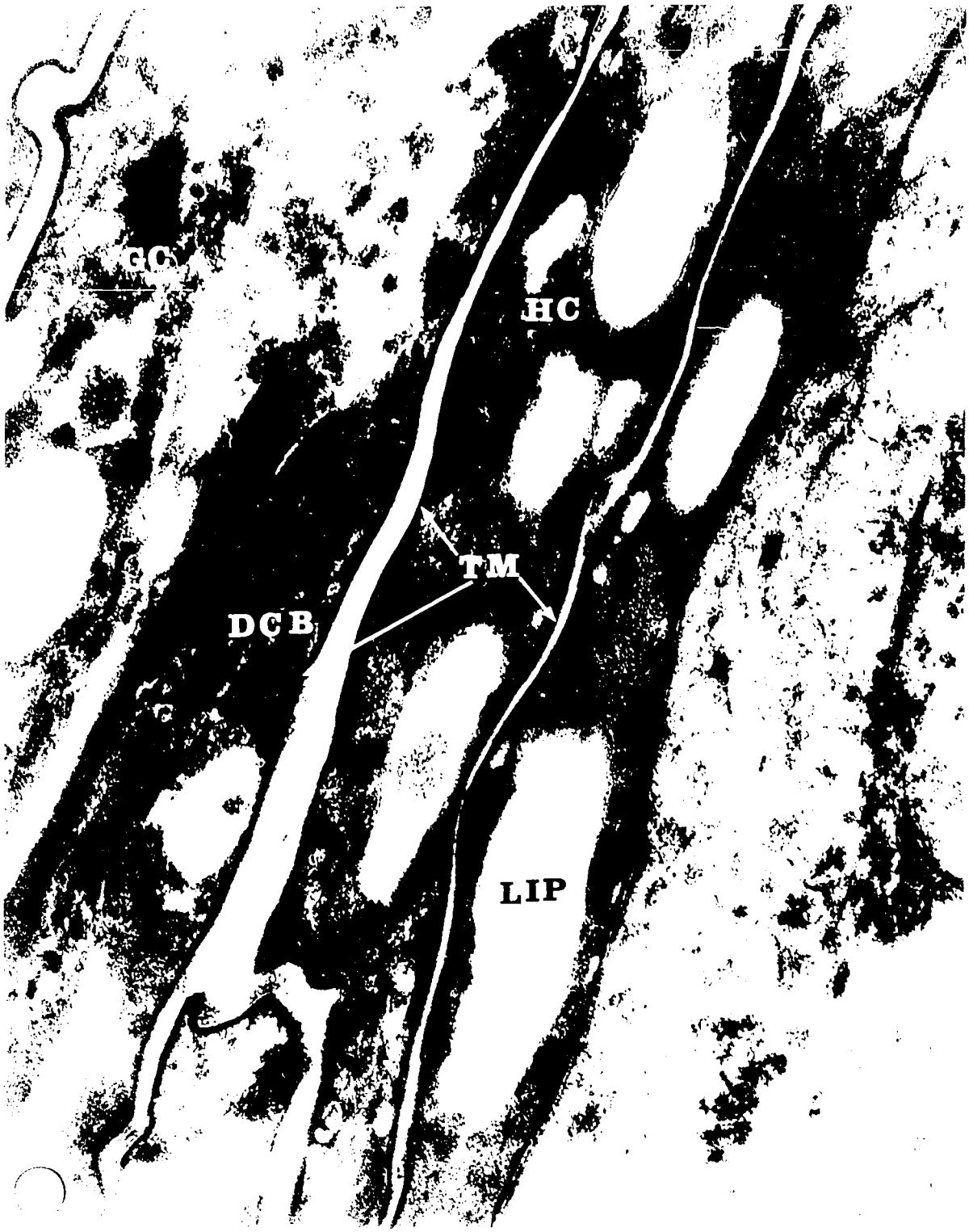

56

Figure 57. Six-day chick embryo skin, eight days *in vitro*. The picture shows two horny cells (HC) and a granular cell (GC) at the left. The plasma membranes of both the granular and the horny cells have greatly increased in thickness. Both keratin fibrils and KH granules are condensed mainly at the periphery of the granular cells (DCB), and this pattern remains in the two horny cells shown in this picture. The central electron light zones of the horny cells are believed to be occupied by lipid (LIP). Epon.

X 59,200


GC
HC
TM
DCB
57


GC
HC
TM
DCB
LIP

(g) Six-day skin, in vitro with excess vitamin A.

It has been previously shown that in the presence of excess vitamin A in the culture, the epidermal cells undergo metaplastic changes to form mucous secreting cells (Fell and Mellanby, 1953; Fell, 1957; Fitton-Jackson and Fell, 1963). Many attempts have been made during this investigation to reproduce these results. Unfortunately, none of them was entirely successful. Although keratinization of many of the skin cultures was prevented, a complete mucous metaplasia was not observed, irrespective of the amount of vitamin A present in the medium (5 to 40 i.u. vitamin A/ml.).

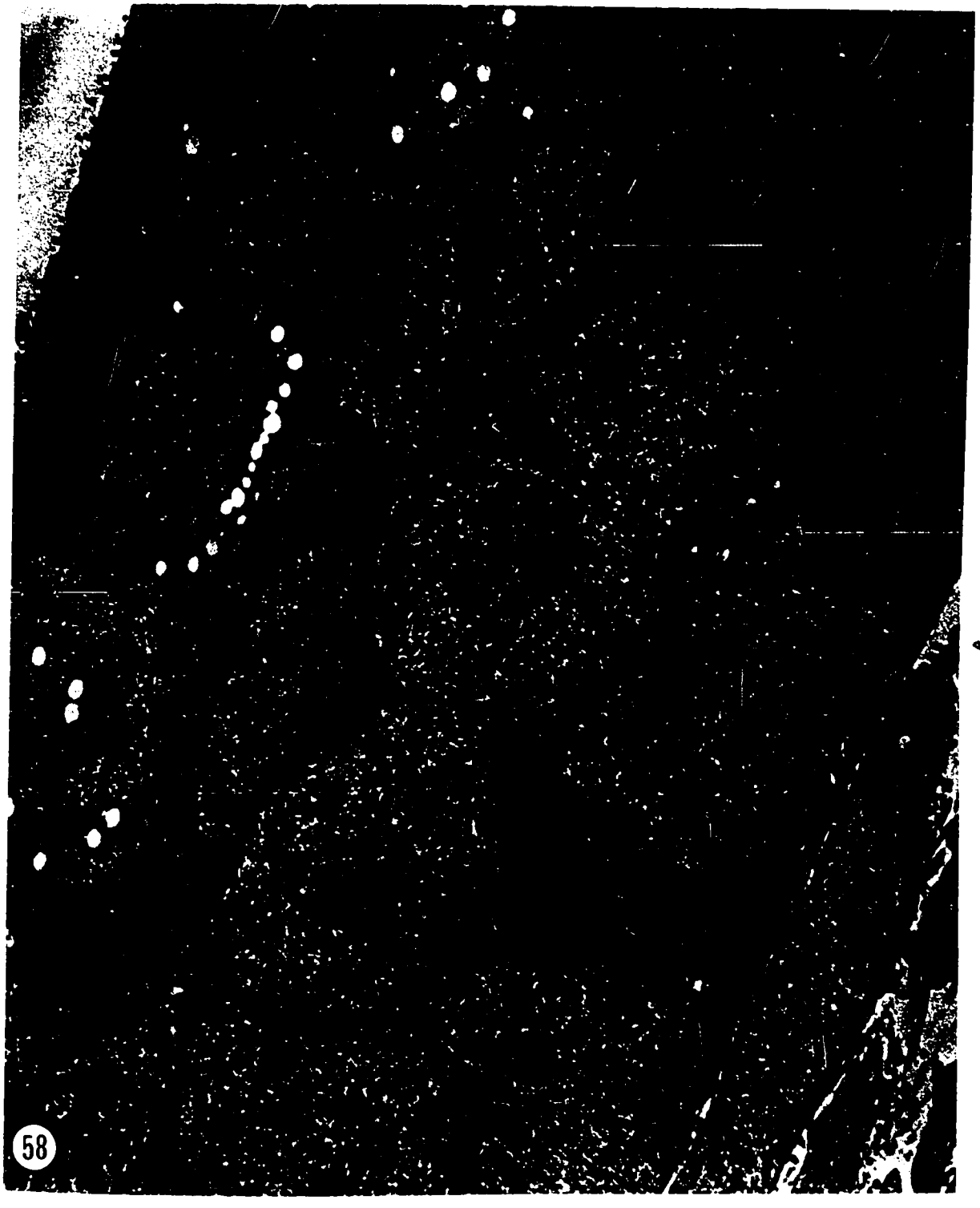
After eight days in culture the epidermis consisted of a layer three or four cells thick (Fig. 58). The basal cells were columnar in shape and had quite prominent RER. Many of the cisternae of the cells were distended with finely granular material. Mitochondria were more concentrated toward the basal region of the cell and many mitochondrial granules were observed. The GA was juxtanuclear in position. Dense granules, believed to be lysosomes, and some myelin figures were also observed. The basement membrane was not so irregular or serrated as in the controls. Collagen fibrils were present adjacent to the basement membrane.

The intermediate cells, cuboidal in shape, had a similar structure to the basal cells. Some fibrils were found both in basal and intermediate cells.

The cells of the superficial layer were usually columnar in shape. A few microvilli were present on the free edges (Figs. 58, 59). The membranesof adjacent cells were convoluted and in close apposition. Tripartite junctional complexes (Farquhar and Palade, 1963) were found at the apical margin. RER was underdeveloped, by comparison with the

Figure 58. Six-day chick embryo skin, eight days *in vitro* with excess vitamin A. The epidermis remains 3 or 4 cells in thickness, lying against a straight thin basement membrane (BM). The cisternae of the RER are quite prominent and in many cases distended with granular material. The membranes are straight and the desmosomes are not well developed. A single layer of cuboidal peridermal cells (P) is still present. The membranes of adjacent cells are convoluted and in close apposition. Vestopal.

X 5700


58

58

Figure 59. Six-day chick embryo skin, eight days *in vitro* with excess vitamin A. A columnar peridermal cell is shown. It has very scanty RER and a supranuclearly located multiple Golgi. A few clear vesicles in the cytoplasm are believed to be lipid granules. Some fibrillary material can be seen in the background but no mucous granules are observed. Vestopal.

X 20,570


59


MV
CCM
59

controls. However, multiple Golgi zones were observed. A few clear vesicles were believed to be lipid droplets. Fine filaments and ribosomes were also present.

The nucleus was normal, with indentations. Nucleoli were found in some cells.

Judging from the convoluted contour of the superficial cells, it was believed that this layer of cells might, in fact, be derived from the periderm of the six-day old chick skin.

5. Mucous Metaplasia of Hamster Cheek Pouch Epithelium.

(a) Normal cheek pouch epithelium.

The fine structure of normal hamster cheek pouch epithelium has been reported by Albright and Listgarten (1962) and is very similar to the structure of epidermis. The tissue had only thin intercellular spaces, and well developed desmosomes. Keratin fibrils were prominent in the spinosum and granulosum. In addition, typical MCG and KH granules were also observed. Horny cells were usually several layers thick.

The most characteristic feature of the cheek pouch epithelium was the presence of large numbers of tight junctions between cells of the basal, spinous and granular layers (Figs. 60, 61). They might be found between flat areas of cell membrane, microvilli, extentions of desmosomes, or quite often between cytoplasmic fingers and the surfaces of adjacent cells. The cytoplasmic fingers were quite often found indenting the adjacent cells. When these were cut transversely the picture shown in the Figure 61 resulted. These have been called "double-walled cytoplasmic vacuoles" (Albright and Listgarten, 1962; and Listgarten, Albright and Goldhaber, 1963). The use of this term

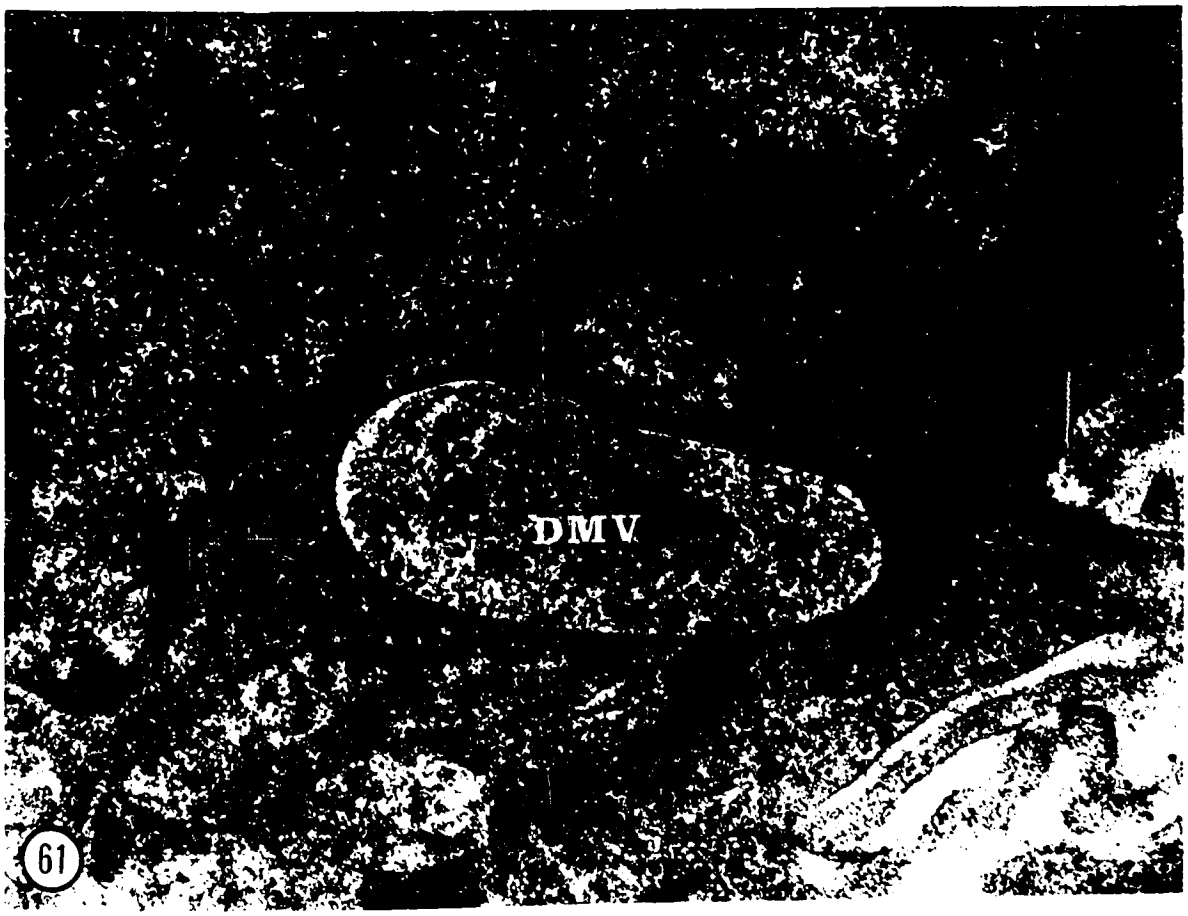
Figure 60. Normal hamster cheek pouch epithelium, showing portions of several spinous cells. They have rather prominent desmosomes. The most characteristic feature of this epithelium is the frequent presence of tight junction (TJ) between cells. Epon.

X 31,000

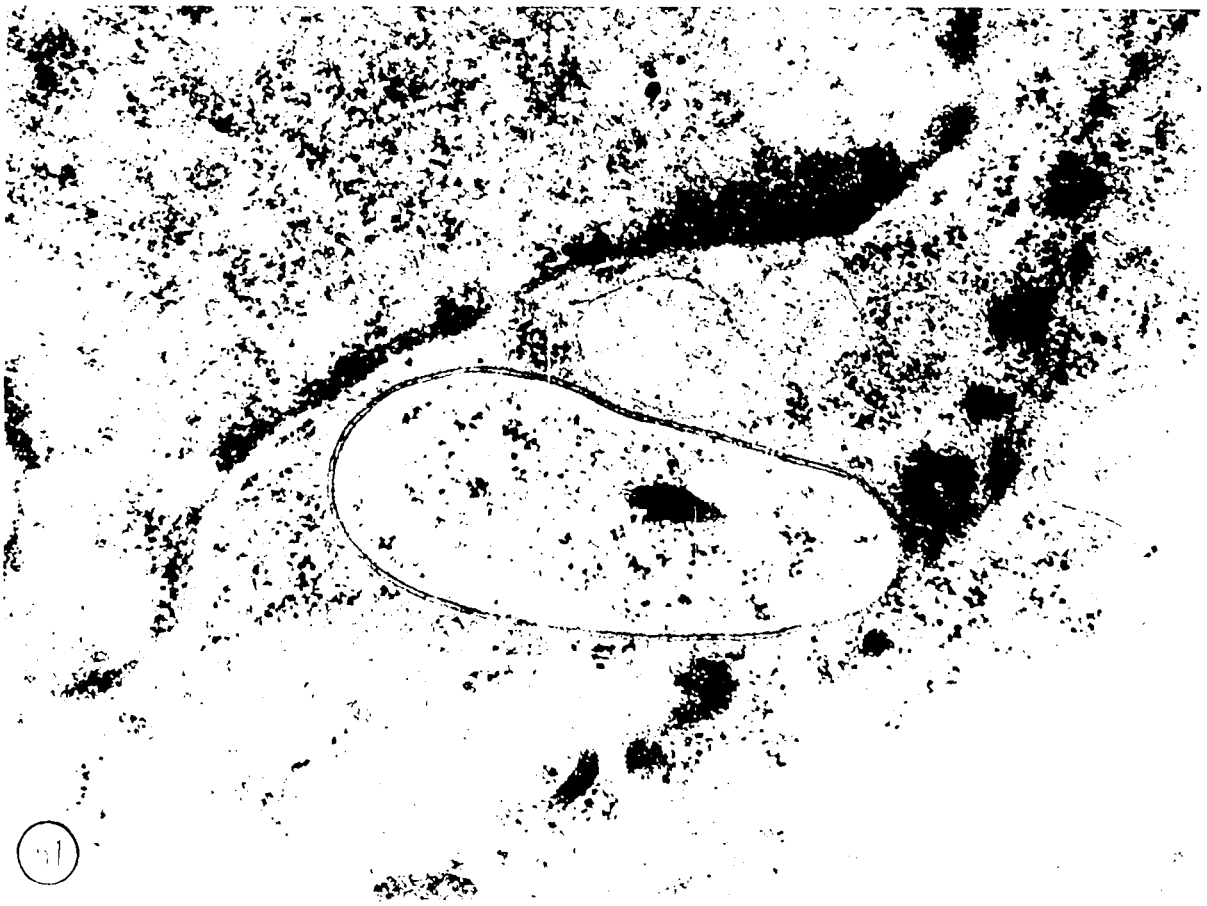
Figure 61. Normal hamster cheek pouch epithelium. The "double-membraned vacuole" (DMW) is another prominent feature of hamster cheek pouch epithelium. Epon.

41,000


TJ
60


DMV
61

60



61

probably reflects the relatively poor resolution of their pictures, in which the central dense lamella of the tight junction of the membrane was not seen.

(b) Five to ten days after vitamin A pellet implantation.

The epithelium had lost its cornified layer five to ten days after vitamin A implantation. This was usually accompanied by some inflammation, and polymorphonuclear leucocytes and macrophages were common in the epithelium. The intercellular spaces were marked widened, and often filled with finely granular material (Fig. 62). Microvilli were common in the intercellular gaps and desmosomes were still common, although in many cases they appeared disrupted (Fig. 63). The gap between two attachment plaques of the desmosomes had been widened. Tight junctions were still common and the "double-walled vacuoles" were found here-and-there in the cytoplasm.

The general arrangement of the epithelium was of squamous type. However, the basal cells were usually columnar in shape (Fig. 64) and rested on a rather tortuous basement membrane. They were held by well developed hemi-desmosomes. There was little fibrillary material to be seen in the basal cells.

The cells above the basal cells ranged from cuboidal to squamous. Keratin fibrils were still prominent but had decreased by comparison with the controls. Keratohyalin granules were not observed.

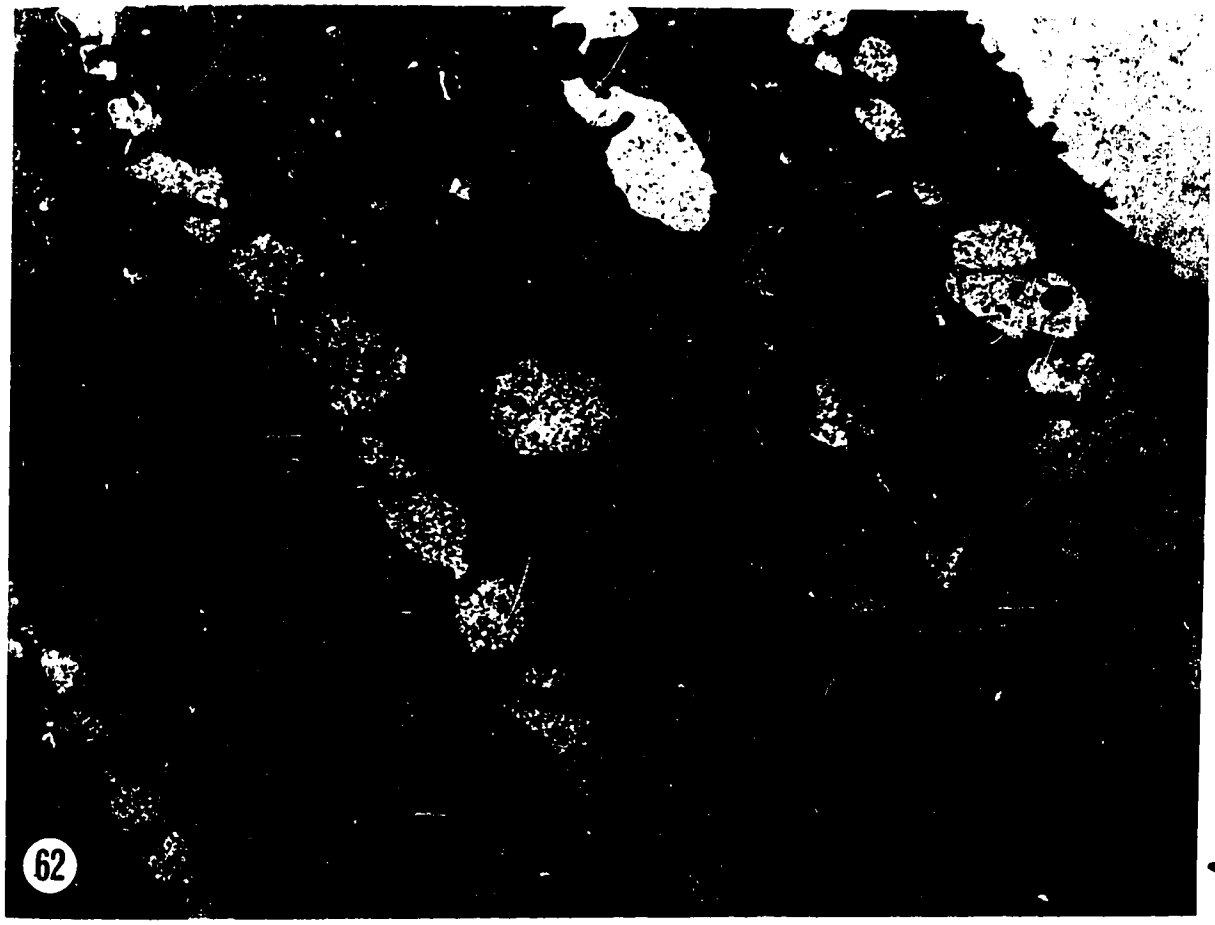
MCG with central lamellae could still be seen (Fig. 65), but they were less numerous than in the controls. In addition, another type of vesicle was observed, slightly larger than the MCG, and membrane bounded (Fig. 66). Most of these vesicles were filled with electron light material, which under high magnification appeared fibrillary.

Figure 62. Hamster cheek pouch epithelium, five days with vitamin A pellet. The epithelium has lost its stratum corneum, but the keratin fibrils remain quite prominent in the cells. Keratohyalin granules are not observed. The intercellular spaces (ICS) are wide and usually filled with granular material. Microvilli can be seen projecting into the intercellular spaces. Epon.

X 9000

Figure 63. Hamster cheek pouch epithelium, five days with vitamin A pellet. The desmosomes between adjacent cells appear disrupted. The gaps between the attachment plaques of the desmosomes have been greatly widened (arrows). Epon.

X 48,000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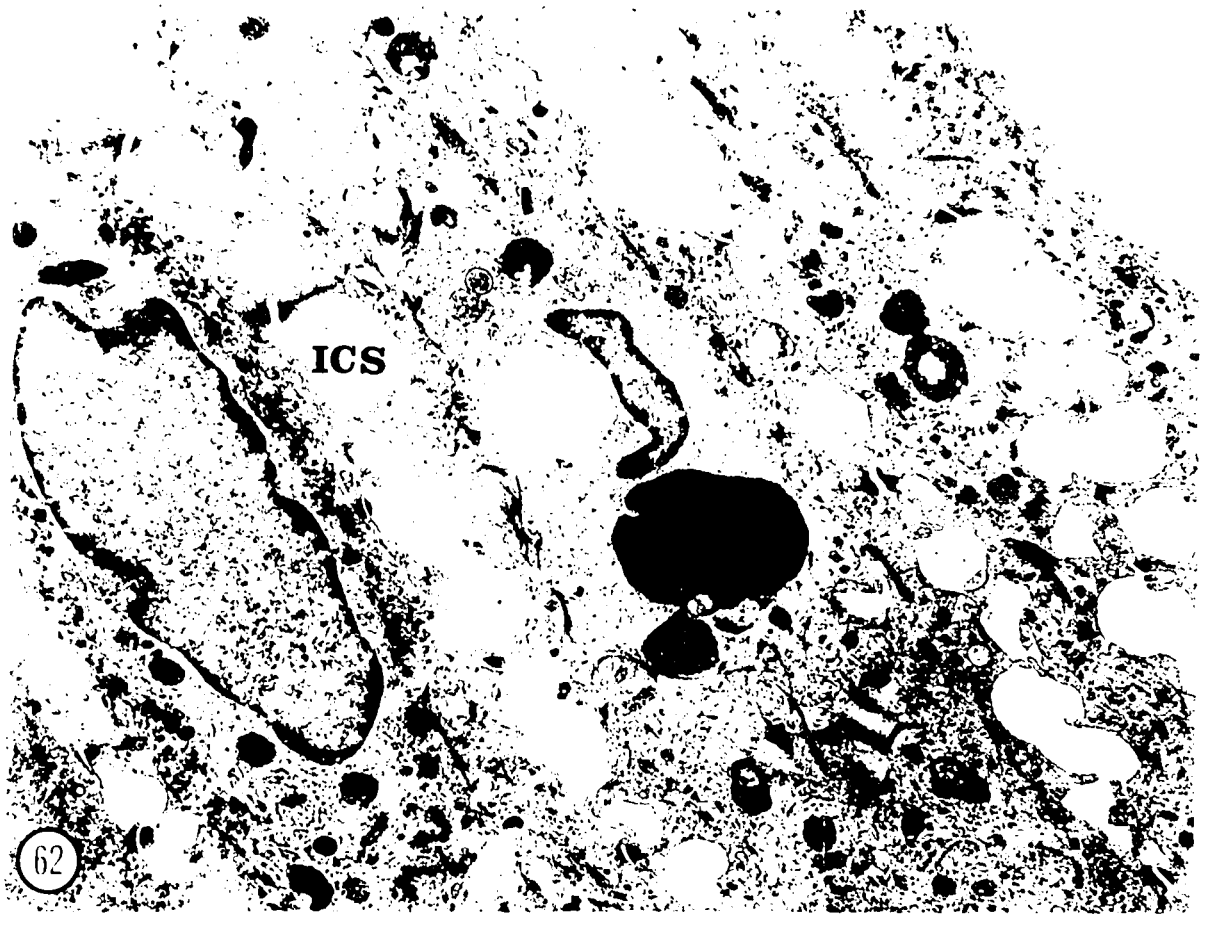
63


ICS
62

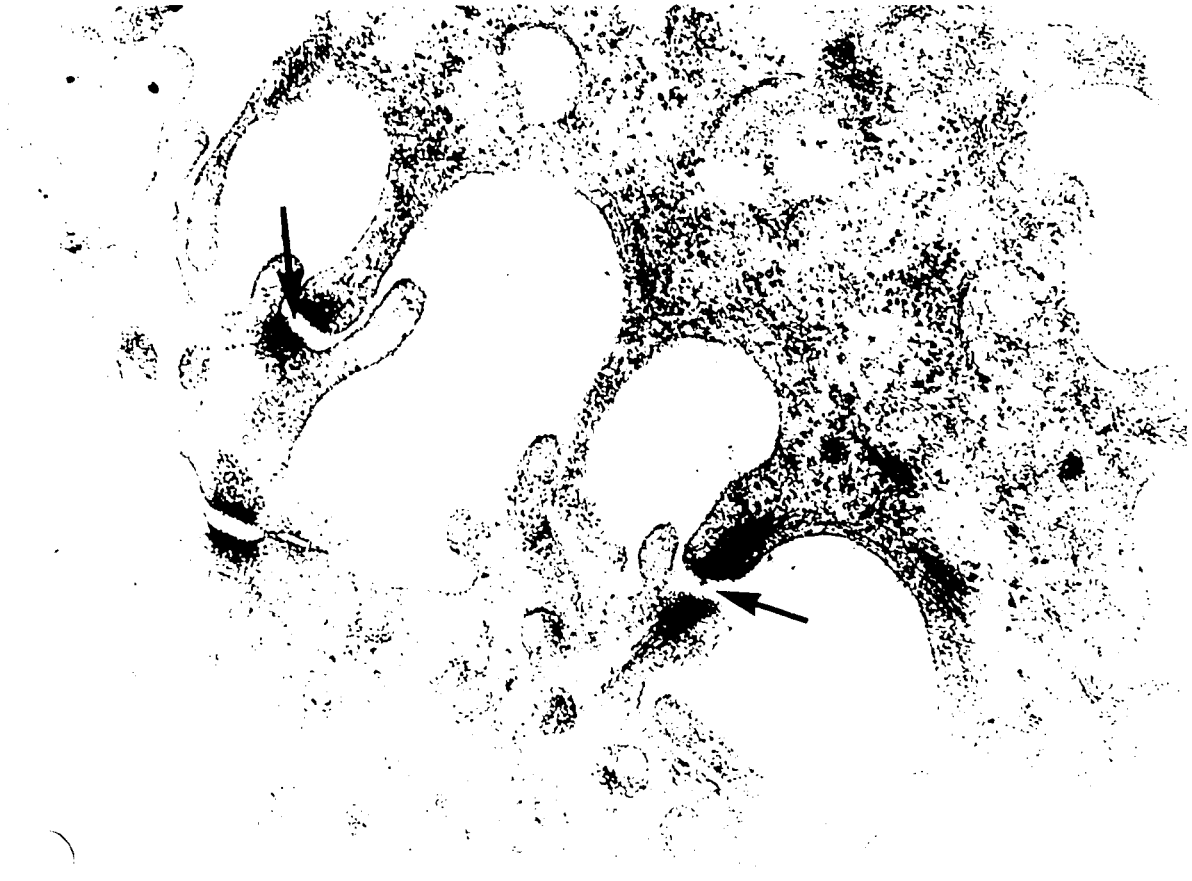


Figure 64. Hamster cheek pouch epithelium, 10 days with vitamin A pellet. The columnar basal cells are lying against a thin basement membrane (BM). Numerous microvilli are intermingled in the wide intercellular spaces between the cells. Epon.

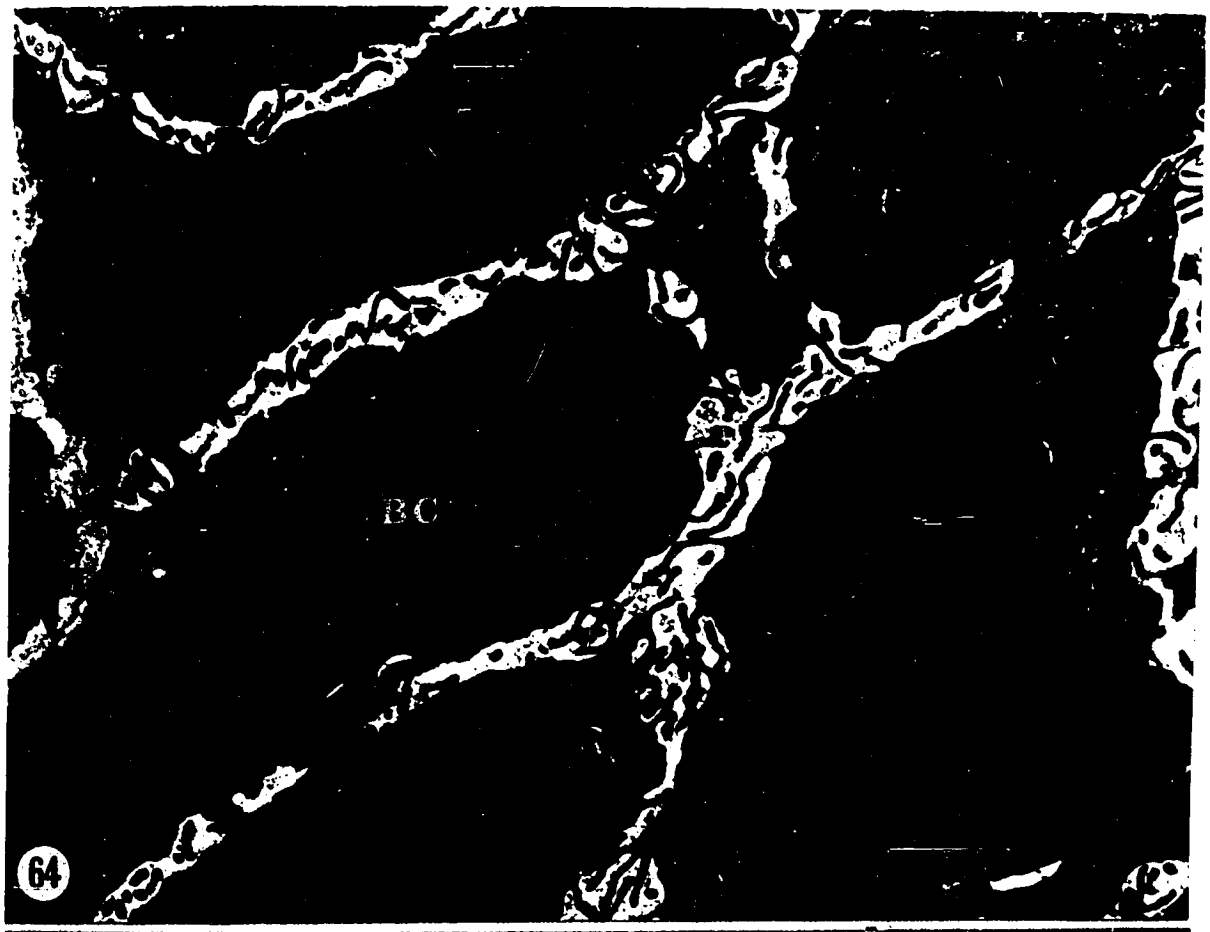
X 12,000

Fig. 65. Membrane-coating granules in hamster cheek pouch epithelium treated with a vitamin A pellet for 10 days . The central laminations of the MCG can be seen. Epon.

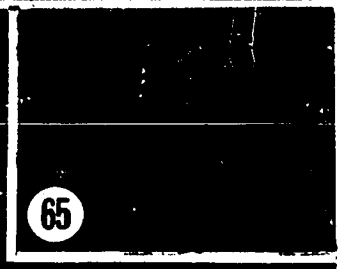
X 127,500

Fig. 66. Hamster cheek pouch epithelium, 10 days with vitamin A pellet. Portion of a squamous superficial cell showing a peculiar type of vesicle. They are membrane bounded and larger than the MCG. Most of the vesicles are filled with both electron dense and light material which may appear in various proportion. Epon.

X 82,500

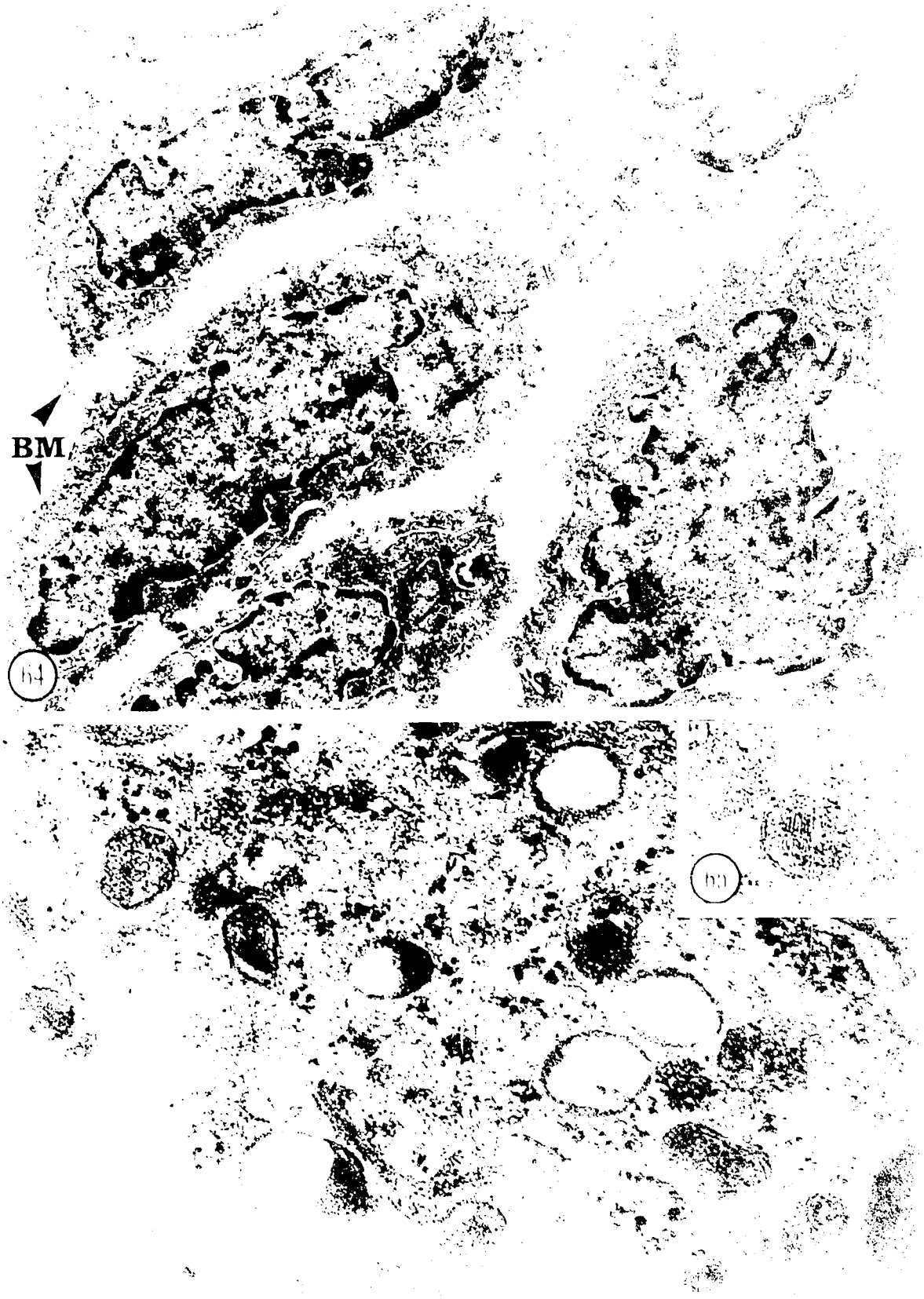


64



65

66


BM
h4
h1

However, not infrequently some dense material could be seen at one end or forming a lining, of the vesicles (Fig. 66). Lamellae, similar to those of MCG could sometimes be observed in the dense portion (Fig. 66). The membranesof RER were prominent in all cells. The association of the vesicles with the GA could be traced.

(c) 15 days after vitamin A pellet implantation.

The epithelial cells were several layers thick. They were composed mainly of cuboidal cells (Fig. 67). The intercellular spaces had become markly reduced and the desmosomes were less numerous and less well developed by comparison with the previous stage.

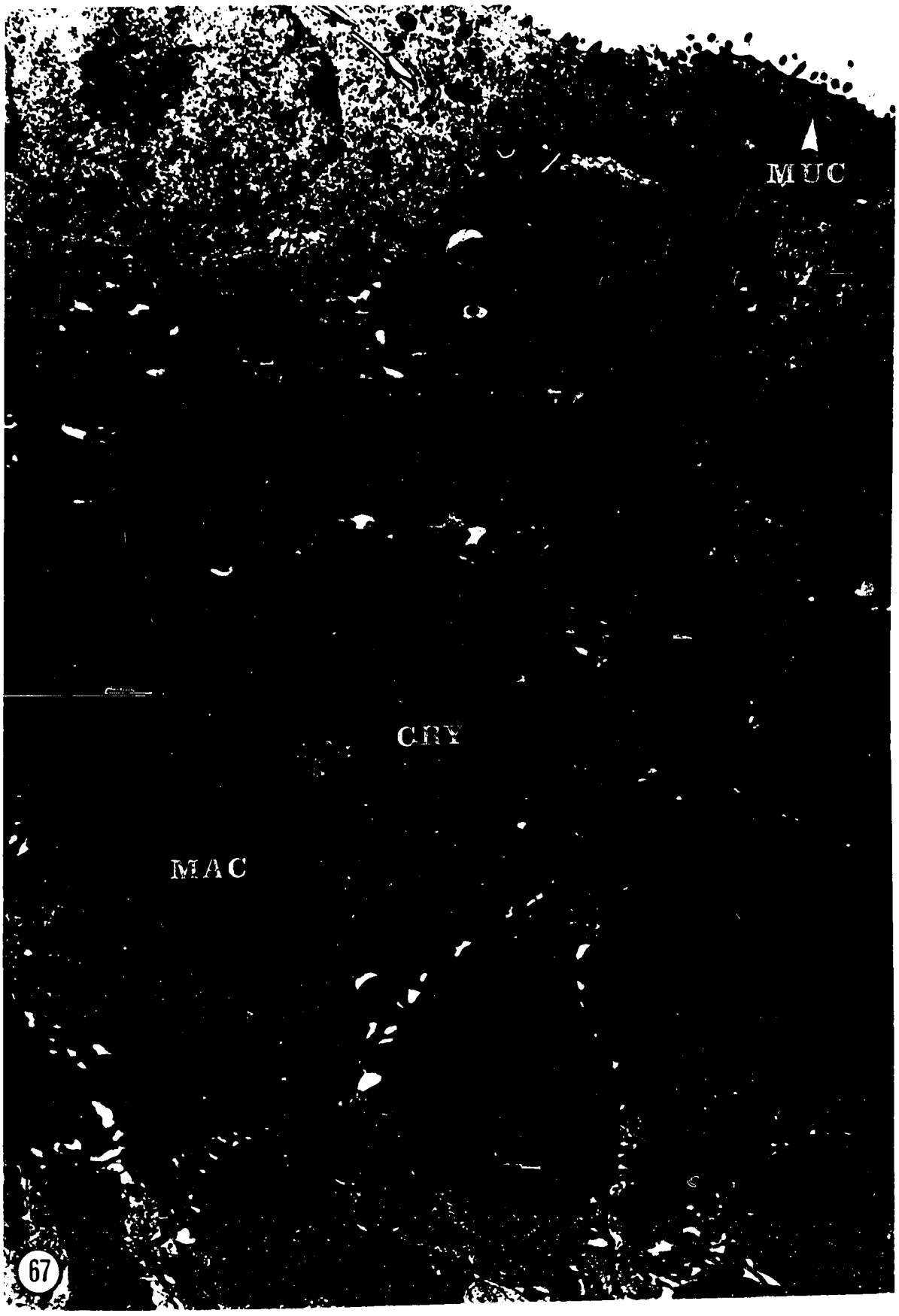
In many cells the keratin had been reduced to a trace, although the RER and ribosomes were still prominent. On the other hand, some cells still possessed considerable amounts of keratin in their cytoplasm (Fig. 67). The nuclei of the cells were usually convoluted and had one or more nucleoli.

Short microvilli were observed on the free edge of the surface layer of cells. A few small mucous granules could be observed in some of the cells (Fig. 67). This was the first indication of mucous metaplasia. These granules appeared to be randomly distributed within the cytoplasm and intermingled with the keratin fibrils of the cells.

In addition, a peculiar type of crystalloid structure which was not observed in the control specimen or in the earlier stages was present at this stage (Fig. 67). These were found in mucous containing cells or, less frequently, in cells deep in the epithelial layers, and also in the macrophages. The details of this crystalloid inclusion will be presented in the next section.

Figure 67. Hamster cheek pouch epithelium, 15 days after vitamin A pellet implantation. The cells are mainly cuboidal and several layers in thickness. The intercellular spaces and the keratin fibrils have become markedly reduced. However, the RER and ribosomes are still prominent. A few small mucous (MUC) granules are present in some superficial cells. A macrophage is also shown in this picture. A peculiar type of crystalloid (CRY) structures is seen for the first time both in the epithelial cells and also in macrophages. Epon.

X 10,000


MUC
CRY
MAC
67

(d) 20-25 days after vitamin A pellet implantation.

A variety of changes in the cheek pouch epithelium was observed in these animals. In some regions hyperplasia or inflammatory reactions were observed, whereas, in other regions complete mucous metaplasia was found.

In the completely metaplastic region, the cells were three or four layers thick. A surface layer of columnar mucous cells were seen lying atop two or three layers of cuboidal cells (Fig. 68).

(i) Mucous cells

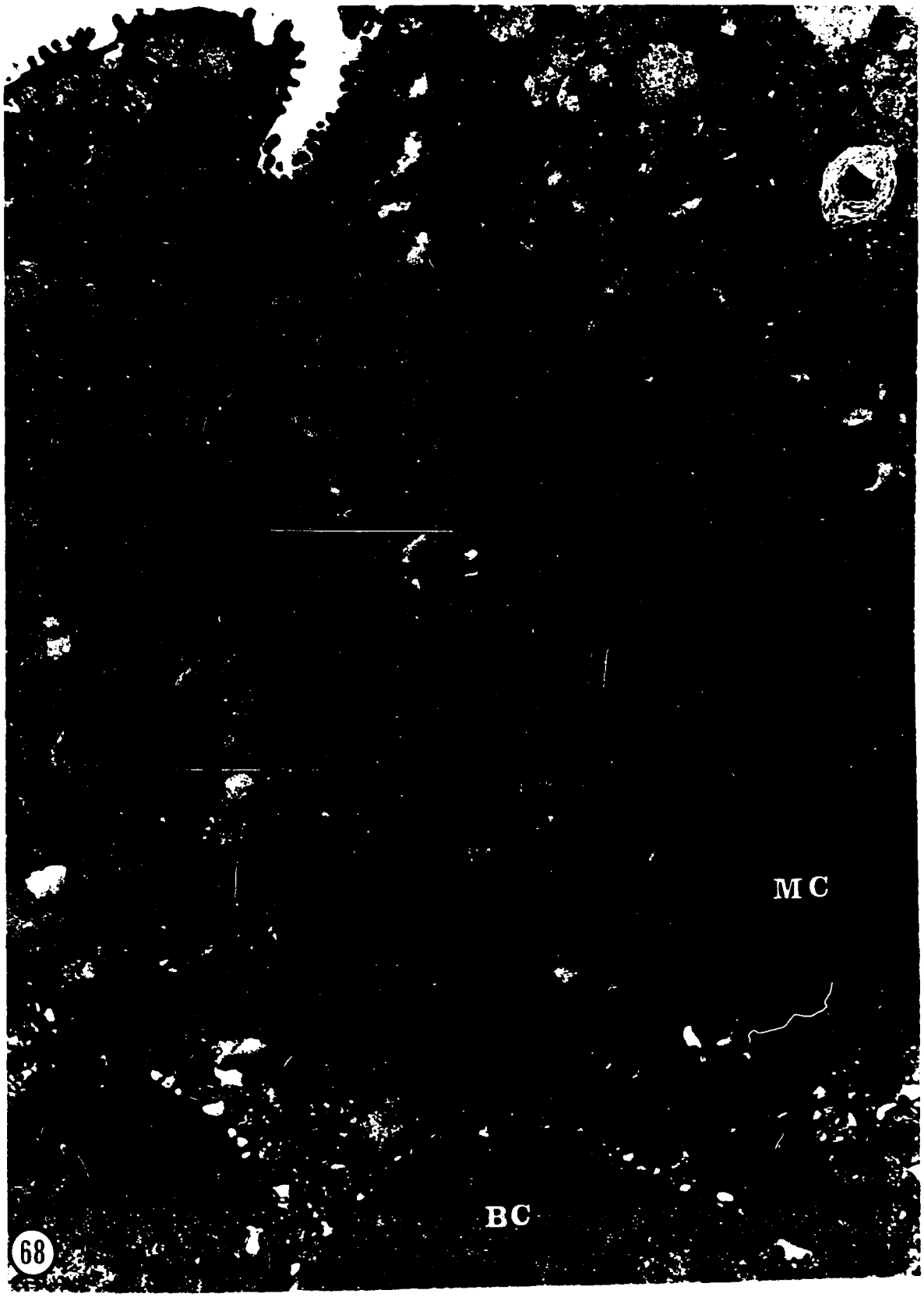
The basally located nucleus was convoluted in outline had one or more nucleoli (Fig. 68). The apices of the mucous cells might be straight or might protrude into the lumen of the cheek pouch (Figs. 68, 69). The free surface of these cells was lined by short microvilli. Under high magnification cell coat material, which was in the form of short fibrillary filaments projecting away from the microvilli, could be readily identified.

The mucous cells contained numerous secretory granules. They were usually concentrated towards the apices of the cells (Figs. 68, 69). The individual granule was usually spherical, completely or partially surrounded by a membrane. The size of the mucous granules ranged from 300 mμ to 1800 mμ. Fusion between granules was commonly seen. These granules were of moderate electron density with only slight variations. Patches of electron dense material could be seen attached to the periphery of some of the granules (Figs. 69, 70). At high magnification spongy, fibrillary material similar to that observed in the vesicles of the earlier stage was observed (Figs. 70, 71, 72).

The GA was well developed and usually located at a supranuclear position, but not necessarily (Figs. 71, 72). They were some-

Figure 68. Hamster cheek pouch epithelium, 25 days after vitamin A pellet implantation. Several typical mucous cells (MC) are shown in this picture. The nuclei of the mucous cells are basally bcated. The cytoplam is packed with mucous granules. Part of two basal cells (BC) is also included in this micrograph. Epon.

X 10,000


MC
BC
68

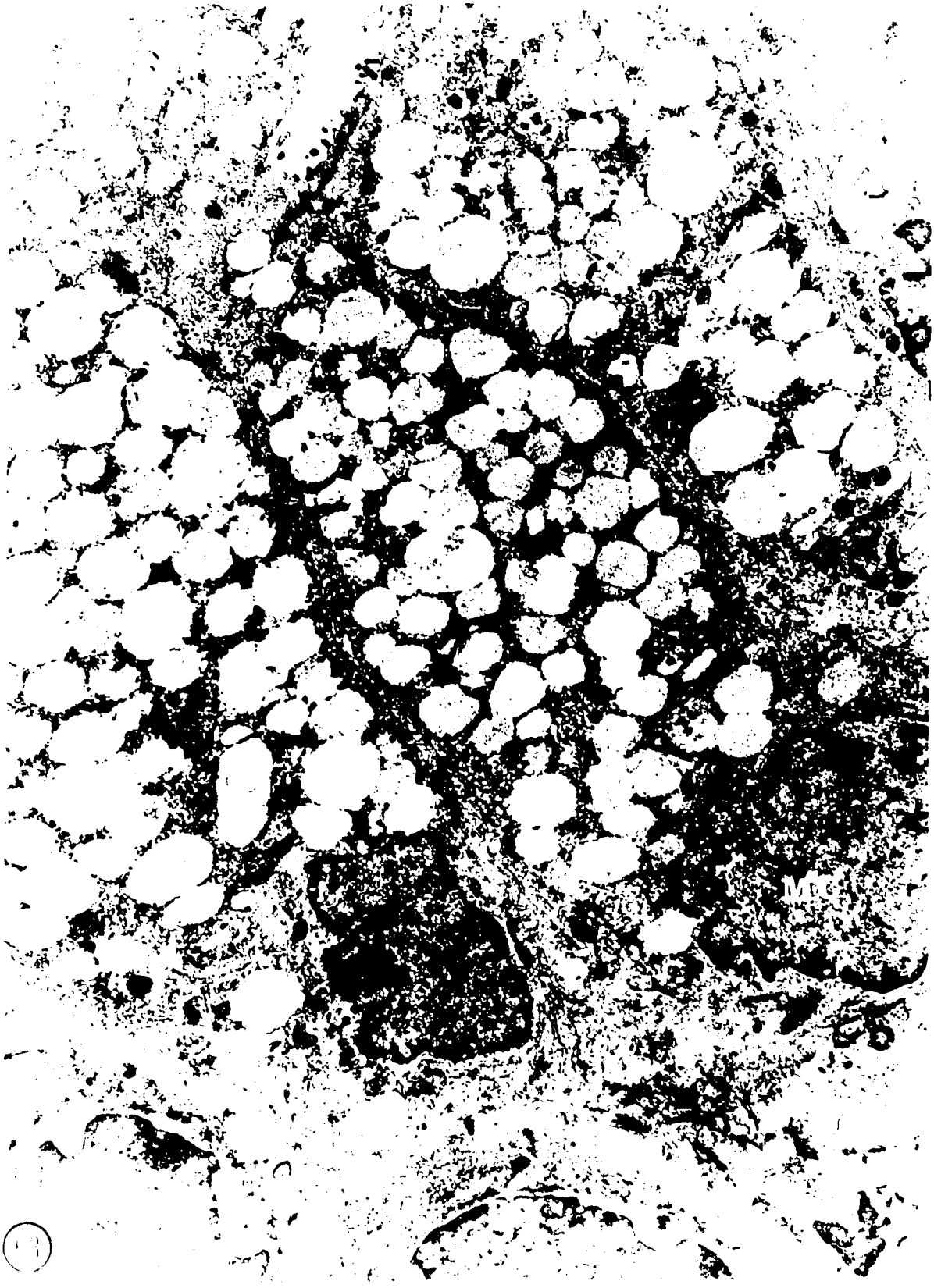
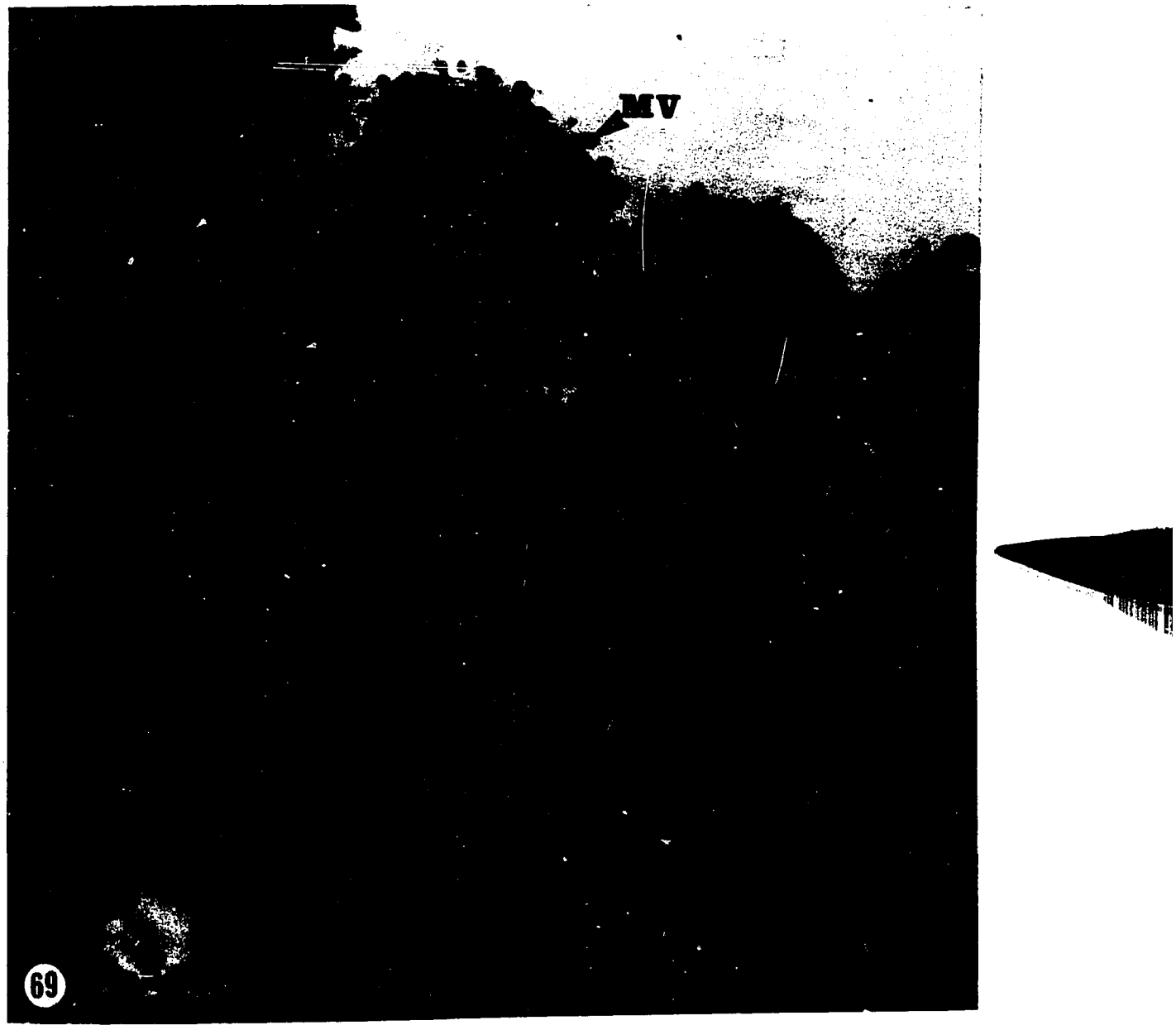


Figure 69. Hamster cheek pouch epithelium, 25 days after vitamin A pellet implantation, showing apical regions of several metaplastic mucous cells. The free surfaces of these cells are lined by short microvilli (MV). A Golgi (G) is shown in one of the mucous cells. Fusion between mucous granules can be seen. The content of mucous granules is fibrillary in nature, with slight variation in density between granules. Patches of dense material can sometimes be seen in the mucous granules (arrows). Epon.

X 14,000


MV
69


MV
G
MUC
69

Figure 70. Hamster cheek pouch epithelium, 25 days after vitamin A pellet implantation. Portion of two adjacent mucous cells, showing dense patches of material (►) at the periphery of the mucous granules (MUC). Epon.

X 22,050

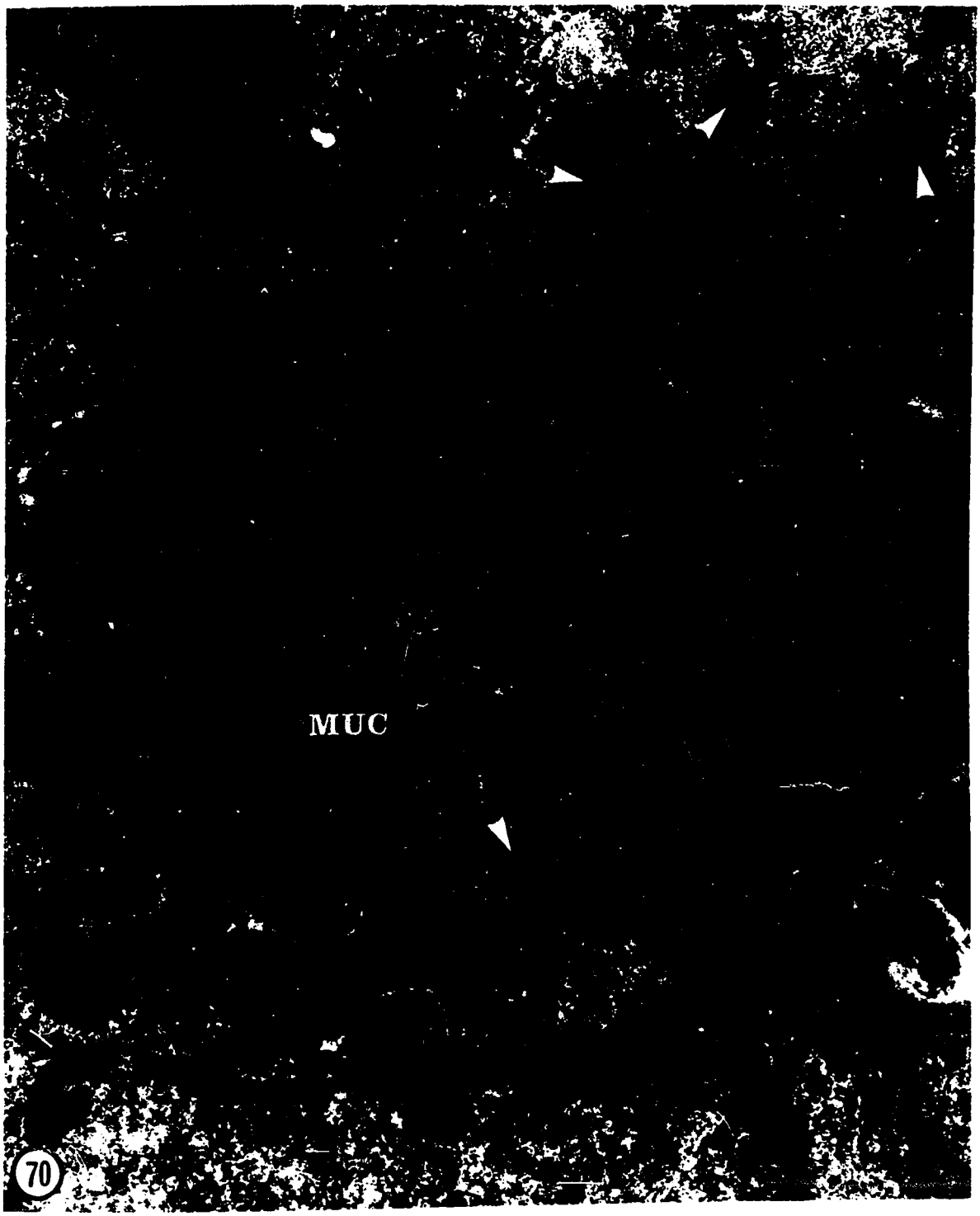
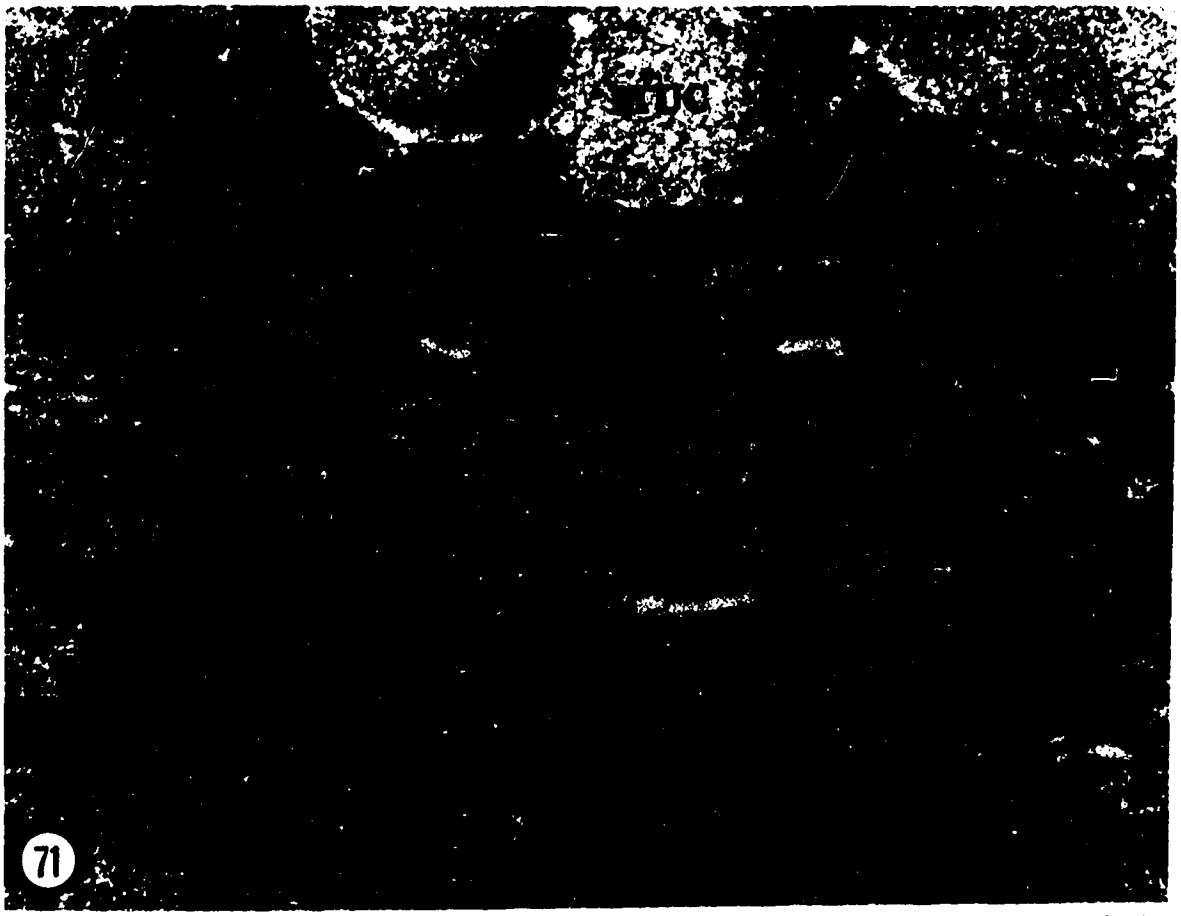

MUC
70

Figure 71. Hamster cheek pouch epithelium, 25 days after vitamin A pellet implantation. The Golgi of the mucous cell is well developed and is closely related to forming mucous granules. Granules of various sizes can be seen within the Golgi zone. Epon.

X 75,000

Figure 72. Golgi from another mucous cell showing a large mucous granule in the Golgi zone. The membrane of the granule is clearly seen. The spongy fibrillary content of the mucous granule is best demonstrated in this case. LIP, Lipid. Epon.

X 75,000



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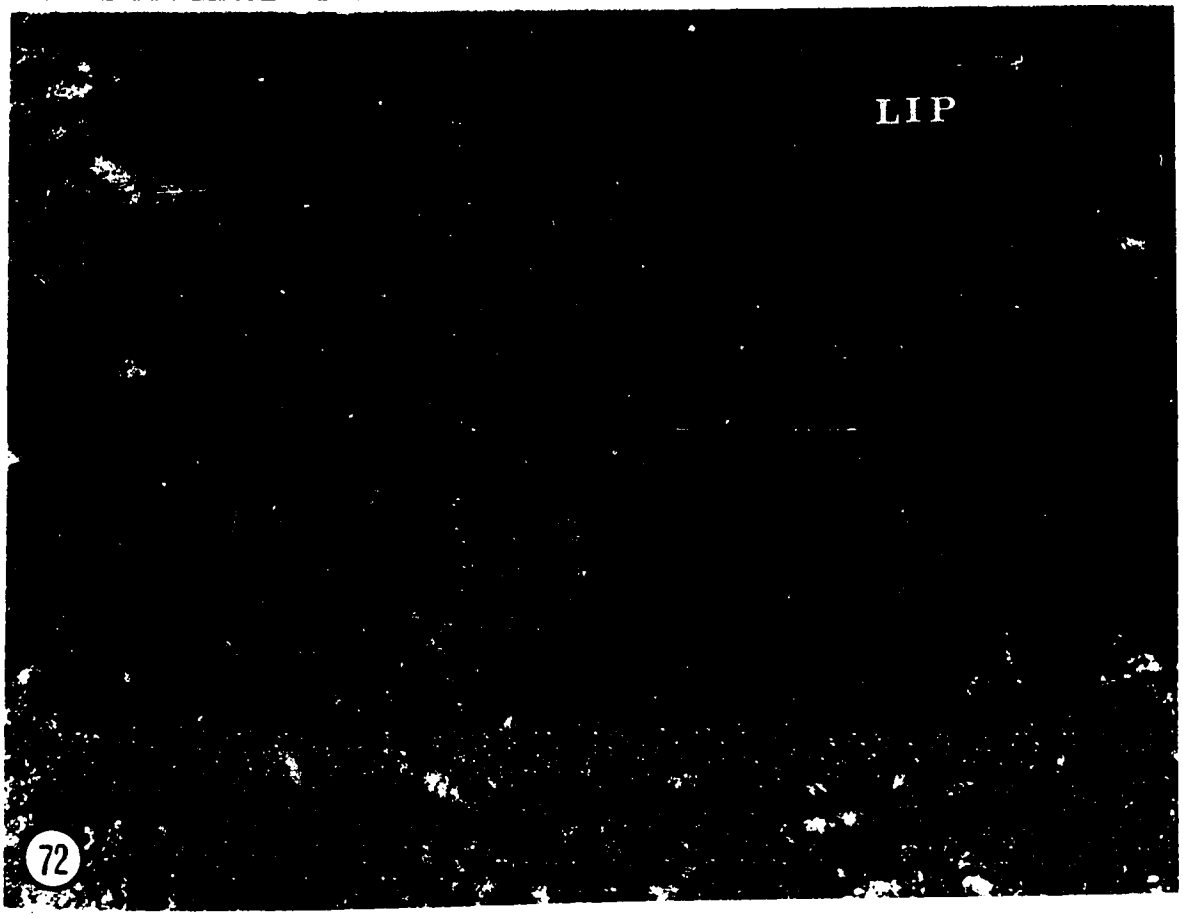


72


MUC
71

times found in a hyponuclear position or as multiple Golgi profiles consisting of parallel lamellae of membranes and many associated vesicles and vacuoles. Mucous granules of smaller calibre were found in the Golgi region (Figs. 71, 72). A well defined membrane limited these mucous granules.

The mitochondria were mainly concentrated in the base of the cell, but a few were found among the granules. Most of the RER was found at the base or periphery of the cell in areas not occupied by mucous granules. Free ribosomes were abundant throughout the cytoplasm.

Lipid droplets and other dense bodies, presumably lysosomes, have also been observed.

A peculiar type of crystalloid body has frequently been observed in the mucous cells and rarely in the basal cells. It appeared as a bar of low electron density and of variable size. The surface contour of the bar was usually straight, giving one an impression that the crystalloid body was rigid (Fig. 73). It ranged from 100 mμ by 1000 mμ to 100mμ by 2-3 μ. When cut perpendicular to the surface a dense line that limited the crystalloid body from the exterior environment could be seen. Within the electron-light crystalloid structure material at higher density could sometimes be observed (Figs. 74, 75). This crystalloid structure was either isolated or surrounded by electron dense material which might be either homogenous or laminated (Figs. 73, 75, 76, 77). Very often several crystalloids might be embedded in a single mass of membrane-bounded dense homogenous matrix and formed a crystalloid-body-complex (Figs. 77, 79).

In other cases granules having the density of lipid granules were seen in association with dense matrix material, and in some cases laminations could be observed (Fig. 75).

Figure 73. A bar-shaped crystalloid body with associated dense material. Very regular striations or laminations (►) can be seen. Epon.

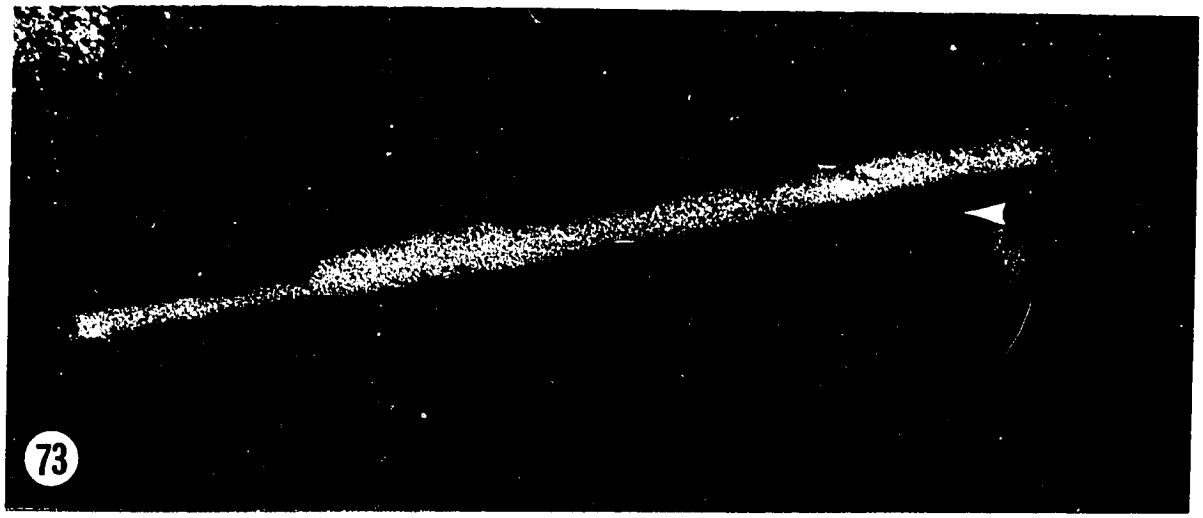
X 64,000

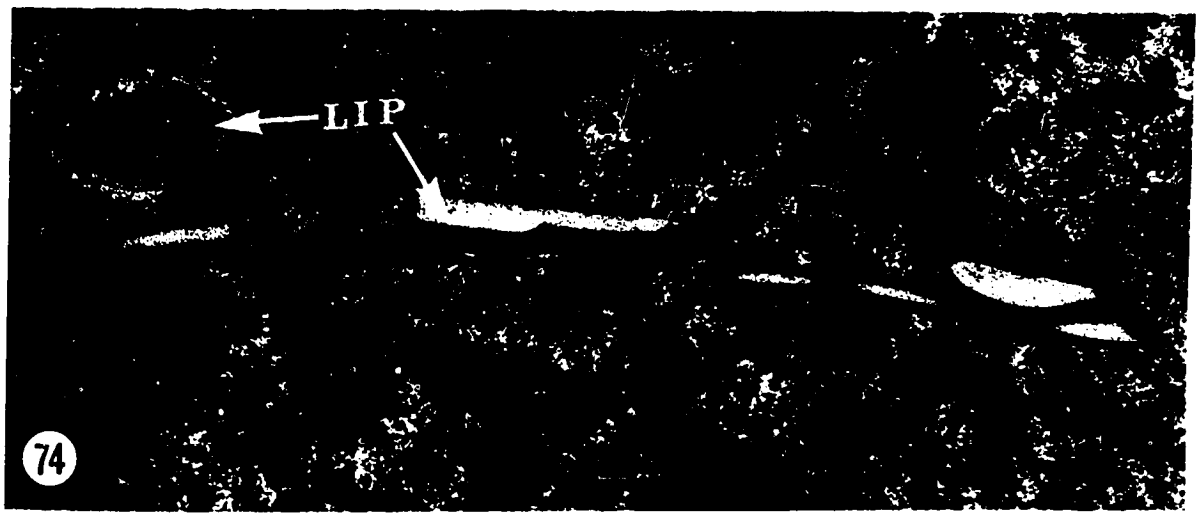
Figure 74. Three similar crystalloid bodies which do not have associated dense material. Electron opaque bands are found in two of the crystalloid structures. They are believed to be lipoid (LIP) in nature. Epon.

X 40,000

Figure 75. A bar-shaped crystalloid structure on the right is partially filled with lipoid material. The one on the left appears to be a big lipid granule, except that it possesses a distinct membrane and a small crescent of electron dense material bearing the regular striations (►) as seen in the crystalloid body in Figure 73. Epon.

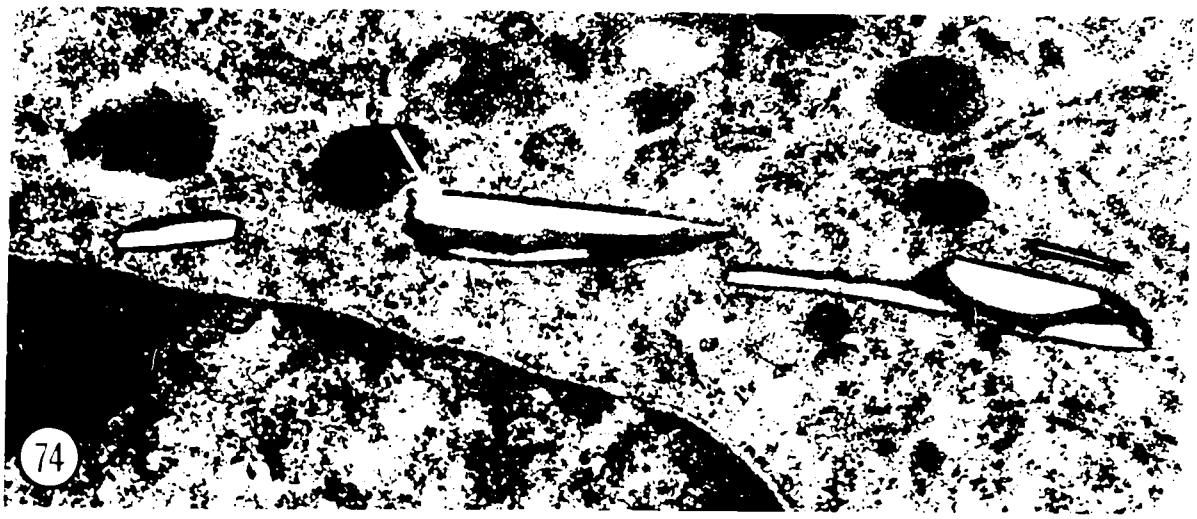
X 82,500


73


LIP
74


LIP
75


73


74


75

Figure 76. A bar-shaped crystalloid body is embedded in a large electron dense mass bearing no striation. Epon.

X 120,000

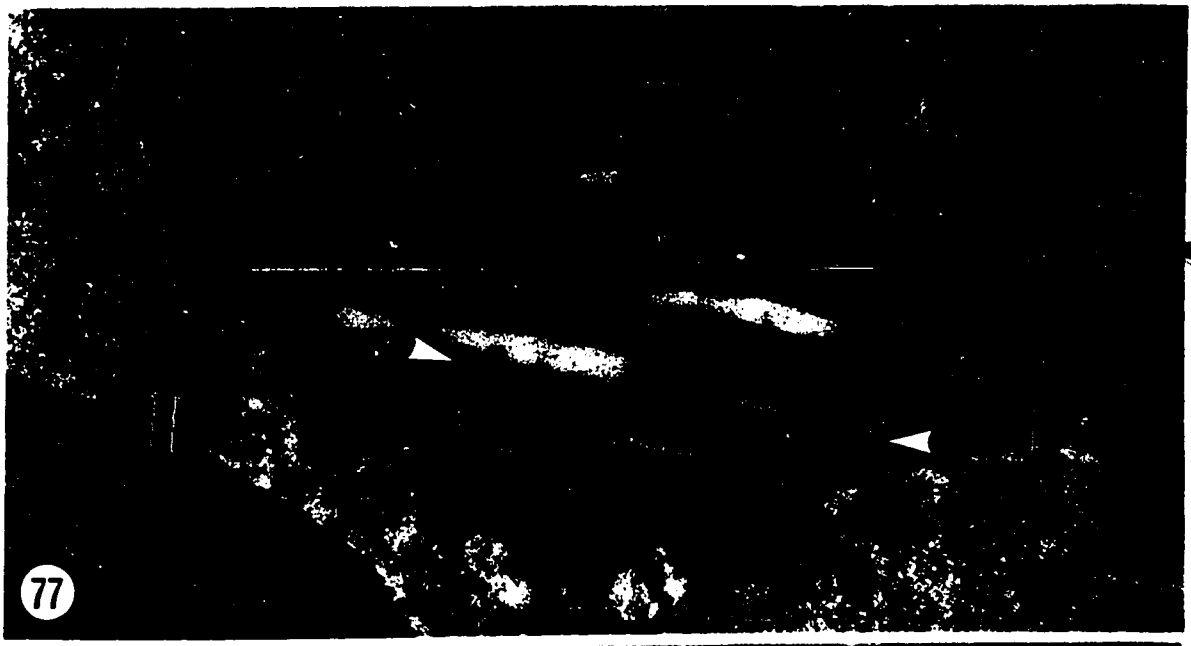
Figure 77. A complex crystalloid body. Several bar-shaped crystalloid bodies are embedded in a big electron dense mass. Regular striations or laminations can be seen in several places.(►). Epon.

X 82,500

Figure 78. Another crystalloid body complex which does not show regular laminations in the matrix. Nearby are three lipid (LIP) granules. Epon.

X 31,500


76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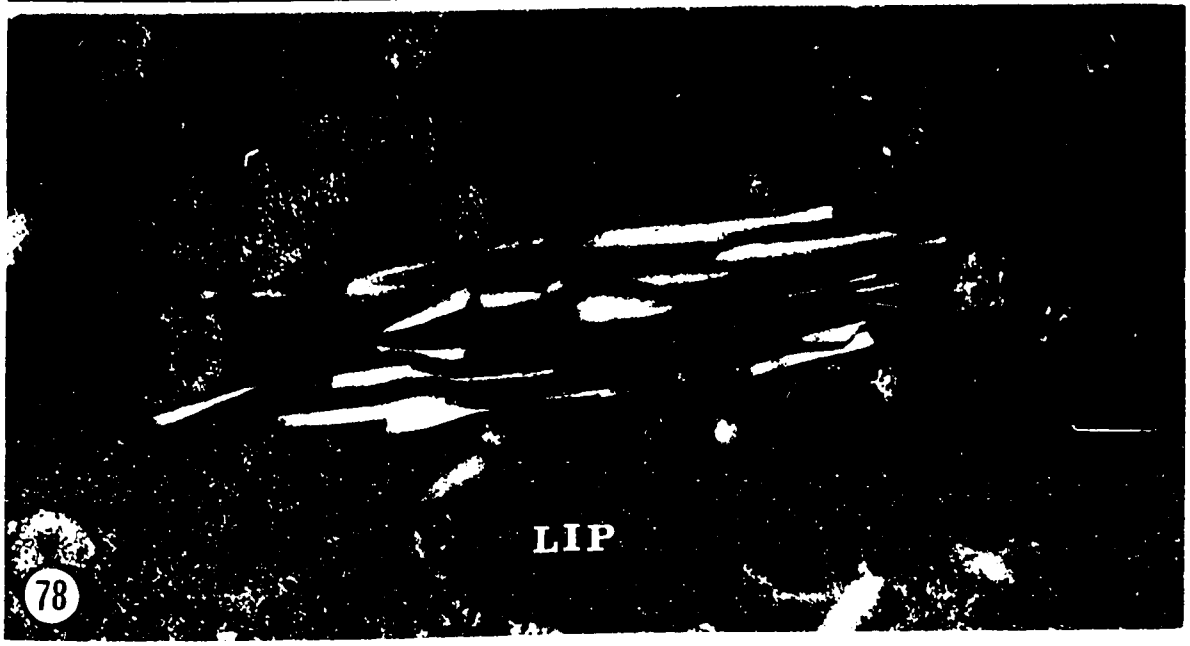

LIP
78

76

77

In addition, a ciliated cell has been observed, the details of which will be presented in a separate section.

(ii) Basal cells

They were usually two or three layers of cuboidal cells. The basal layer lay against the tortuous basement membrane. The nuclei were convoluted, usually with prominent nucleoli. The RER and ribosomes were prominent. Some keratin fibrils were consistently observed. The GA might be multiple and located in a supranuclear position. Occasionally, a few mucous granules might be seen in the layer of cells beneath the mucous cells. Crystalloid bodies were rarely seen in these cells and tight junctions were not commonly seen.

6. Mucous Metaplasia of Mouse Vaginal Epithelium

(a) Vaginal epithelium of the castrated mouse.

Two or three weeks after ovariectomy the vaginal epithelium of the mouse assumed a state of two or three layers of cells (Fig. 79).

The basal cells were usually cuboidal in shape and lay against a tortuous basement membrane. There was a moderate number of ribosomes and relatively few membranes of the RER. A few fibrils were found in the cytoplasm.

The surface cell layer was either cuboidal or low columnar, with a moderate number of short microvilli on the free edge. The GA and vesicles were found in a supranuclear position. Occasionally, a few vesicles with the appearance of mucous granules were also observed (Fig. 80). The plasma membrane between cells was undulating, with desmosomes sparsely distributed.

(b) Estradiol benzoate treated animals.

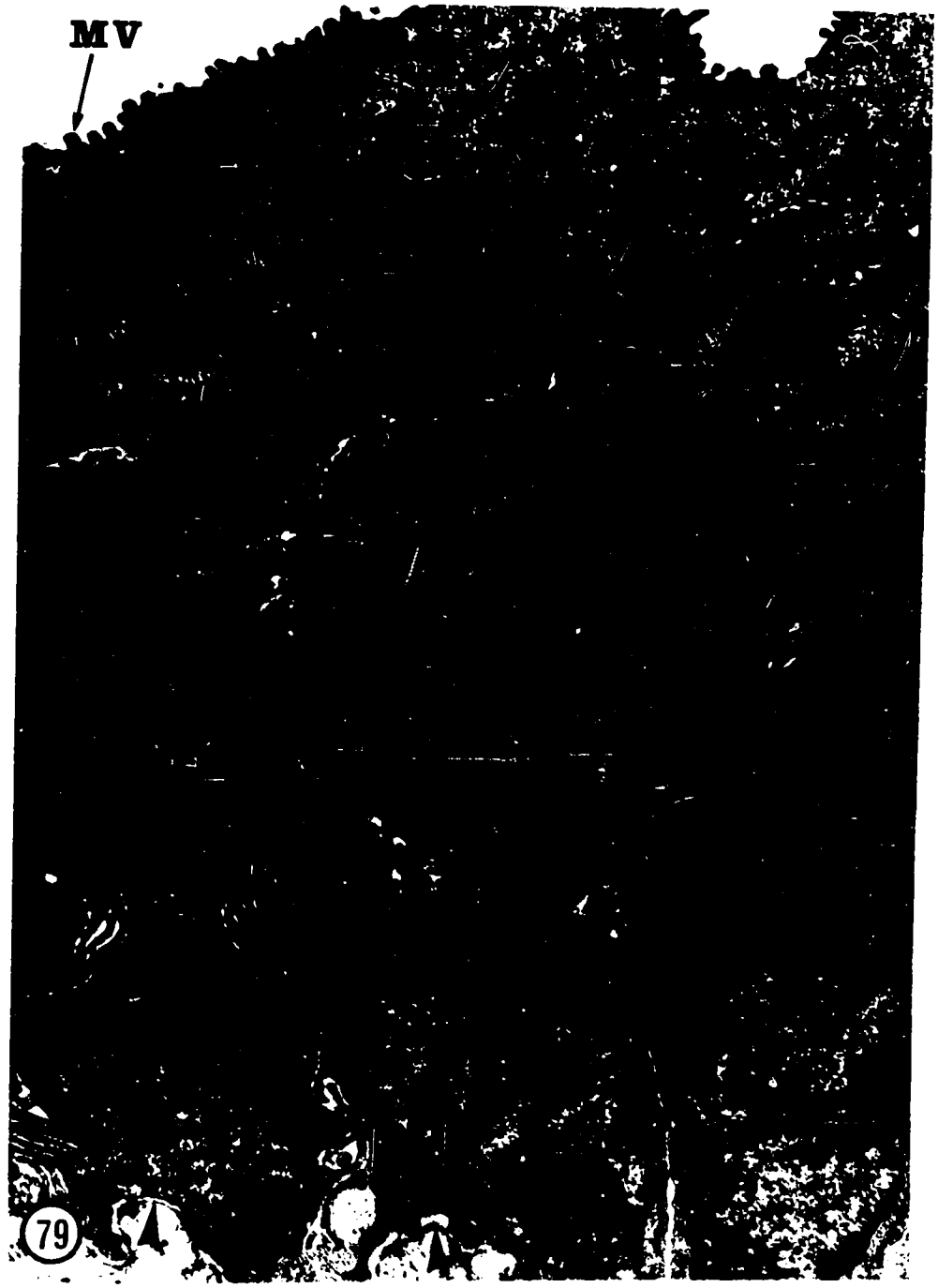
After treatment of the ovariectomized mice with 0.2 μg/per day

Figure 79. Mouse vaginal epithelium, four weeks after ovareictomy. The epithelium is three layers in thickness. The basal cells are cuboidal and lie against a tortuous basement membrane (➤). The desmosomes are not very many in number but are quite well developed. The nuclei are large and usually have deep indentations. A small number of keratin fibrils can be seen in these cells. Short microvilli (MV) are regularly present on the free edge of the surface cells. Epon.

X 11,750

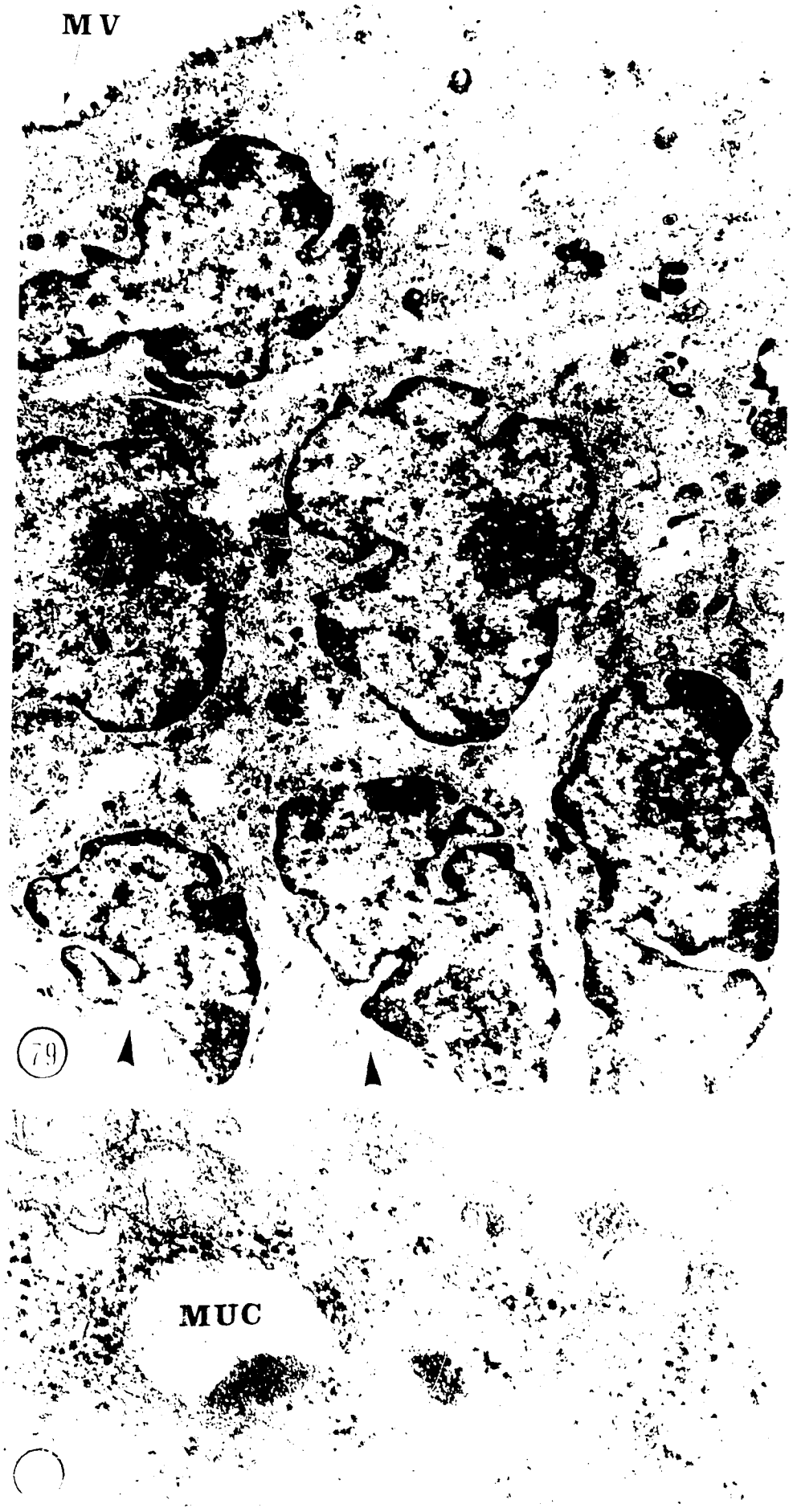
Figure 80. A small portion of a superficial cell showing mucous (MUC) granules in the vaginal epithelium of the control animal. Epon.

X 73,280



79

80


MV
79
MUC

of estradiol benzoate in 0.03 ml. of sesame oil for three or four days, the epithelium became fully cornified. It developed a very thick stratum corneum and stratum spinosum. The latter was packed with keratin and showed very prominent "intercellular bridges". These results are very similar to those reported by Cooper, Cardiff and Wellings (1967) on the effect of estrogen on the vaginal epithelium of immature mice.

(c) Animals treated with progesterone for two days.

When treated with 1 mg/per day of progesterone in 0.1 ml. of sesame oil for two days, the vaginal epithelium of the castrated mouse remained two layers in thickness. Occasional intermediate cells were present. Their nuclei were convoluted and chromatin was usually condensed at the periphery of the nuclei (Fig. 81). The boundary between the surface and the basal cells was tortuous, with cytoplasmic projections intermingled with each other.

The surface epithelium was columnar in shape. The most striking feature was the presence of mucous granules in the cytoplasm. These were more numerous in some cells than in others (Fig. 81). Most of the mucous granules were located in the supranuclear position but occasionally a few granules were found basal to the nucleus. The granules were enclosed in a thin membrane which in many instances was not readily seen. In others the membrane surrounding the granules was definitely incomplete and the contents of the granules appeared to be oozing into the cytoplasm. The mucous granules showed relatively low electron density except at their periphery where some electron dense areas were usually seen (Fig. 81). At higher magnifications the mucous granules were seen to contain numerous filamentous structures.

RER was prominent and the GA was located either supranuclearly

Figure 81. Two-day progesterone. The epithelium is two layers in thickness. The basal cells (BC) are mainly cuboidal, while the superficial mucous containing cells (MC) are mainly columnar in shape. The nuclei of all cells are indented with one or more nucleoli in each of them. The chromatin is condensed to form a chromatin band at the periphery of the nucleus. Mucous granules are found in various amounts in the apical cytoplasm of the superficial cells. Electron dense patches (arrows) can also be seen in the mucous granules. Araldite-Epon.

X 8650


MC
BC
81


81

or lateral to the nucleus. An association which was observed between the mucous granules and the GA will be presented in the later section. Moderate numbers of ribosomes and some filaments were observed in the cytoplasm.

The basal cells, in a single layer, were columnar in shape with a moderate amount of RER. Keratin filaments, sometimes appearing in bundles, were found in the basal cells. The GA was small and no mucous granules were found (Fig. 81).

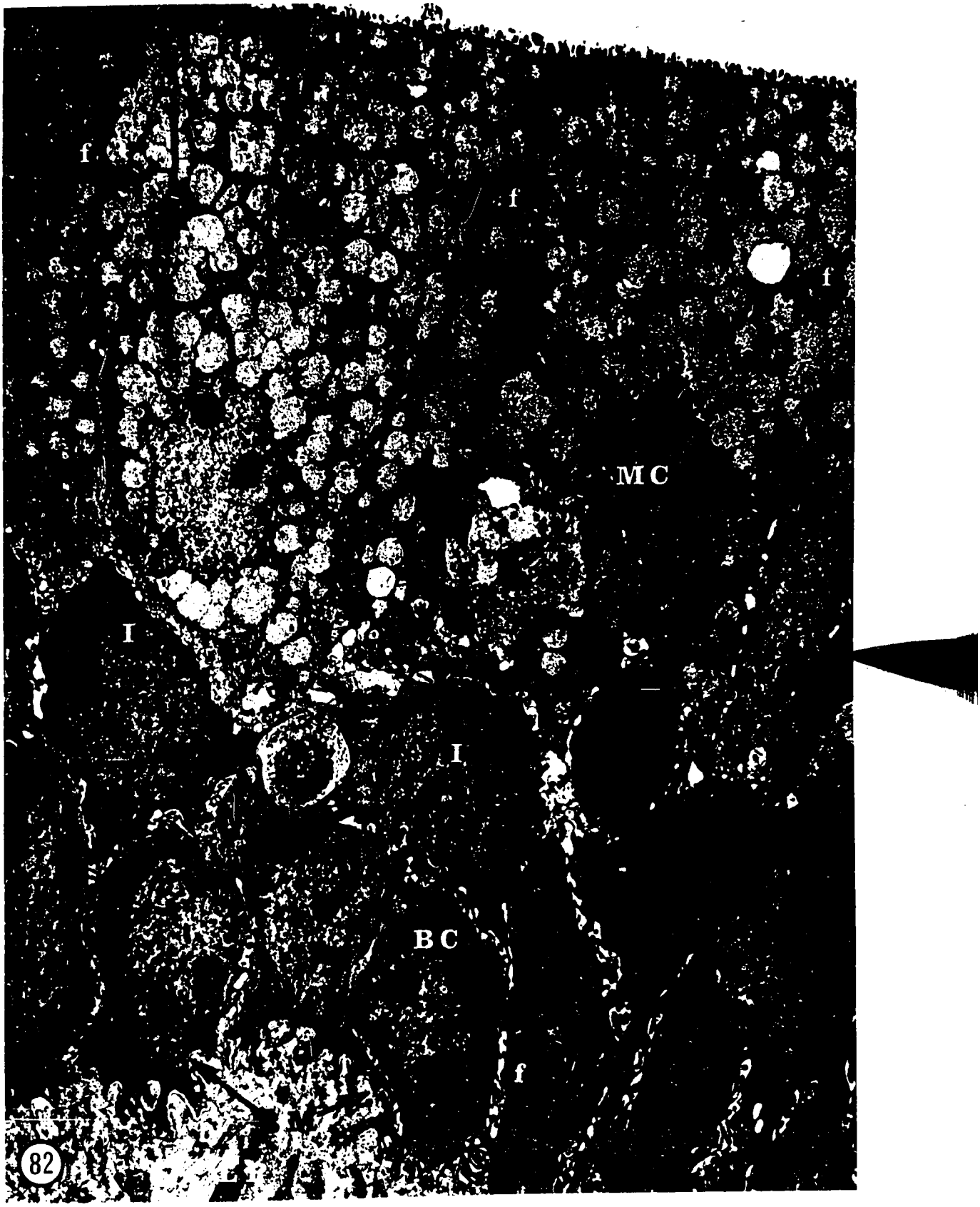
(d) Progesterone for four days or more.

When the animals were treated with progesterone for four days or more, typical mucous secreting cells were seen (Fig. 82). The epithelium was composed of a basal layer, a mucous cell layer and some intermediate cells.

The mucous cells were columnar and had short microvilli on the free surface (Figs. 82, 83). The cytoplasm of the mucous cell was packed with mucous granules. Many of the granules coalesced, as judged by the formation of large continuous mucous compartments in the cytoplasm (Figs. 82, 83). The nucleus was quite often found to be surrounded by numerous mucous granules which, in some cases, appeared to isolate it from the rest of the cytoplasm (Fig. 84). The nuclear membrane was usually indented by, and followed the contours of the mucous granules (Figs. 83, 84). The dense droplets in the mucous granules mentioned in the previous section were also observed in these animals (Figs. 82, 83). The remainder of the granule appeared filamentous (Figs. 83, 84). The GA was usually found lateral to or beneath the nucleus (Fig. 85). Stacks of Golgi membranes and vesicles and mucous granules of various size were prominent in the Golgi zone (Figs. 85, 86).

Figure 82. Four-day progesterone. A layer of typical mucous cells (MC) is shown at the top of the picture. The nuclei are basally located and the apical cytoplasm is packed with mucous granules of various sizes. The cytoplasm of the mucous cells is quite dense and contains considerable amounts of keratin fibrils (f). Microvilli are present on the free surface. Basal cells (BC) are cuboidal in shape and contain moderate amounts of fibrils. Several intermediate (I) cells are also shown in this picture. BM, Basement membrane. Araldite-Epon.

X 3600


f
f
f
MC
I
I
BC
f
82

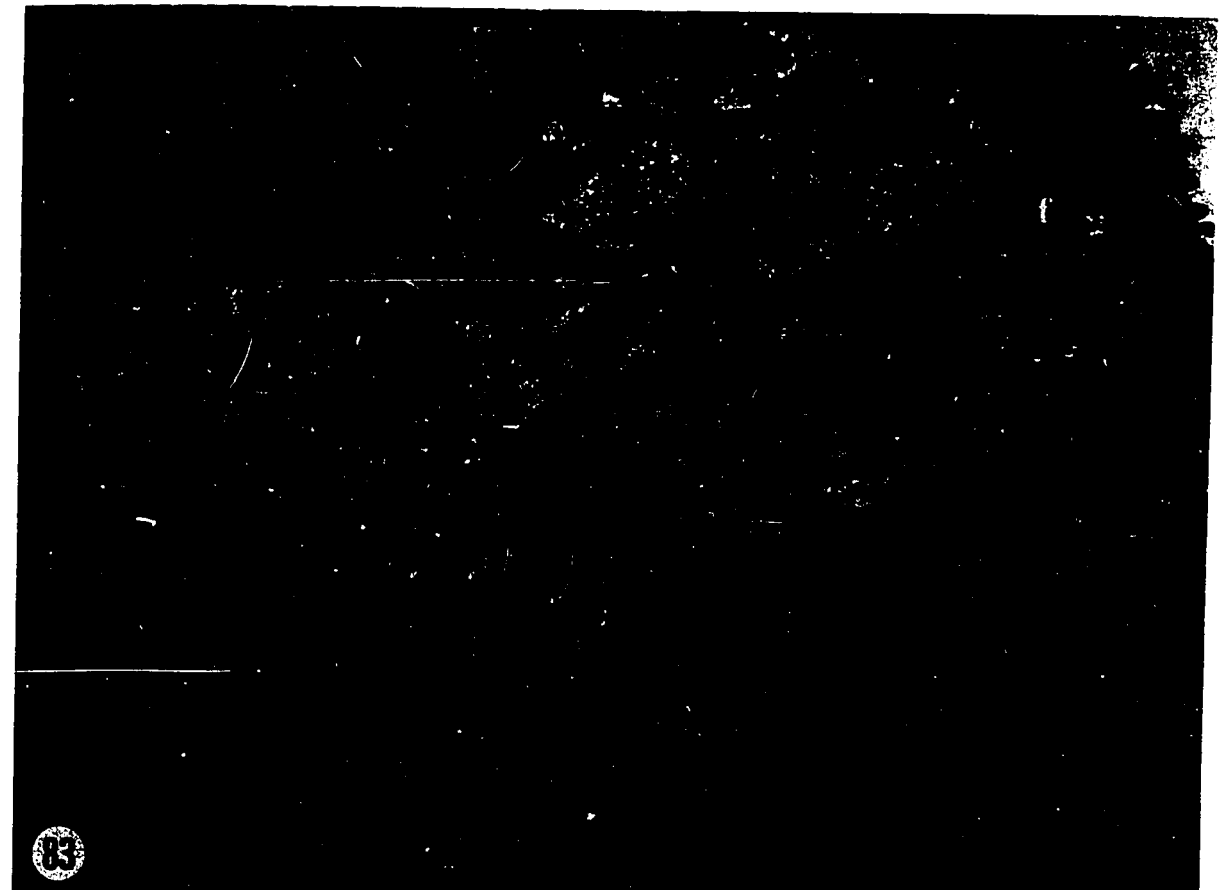

f
f
f
f
M
M
BM
82

Figure 83. Four-day progesterone. Apical cytoplasm of a mucous cell showing large numbers of mucous granules. Spongy fibrillary material is seen in the mucous granules. The RER is prominent between granules, and keratin fibrils (f) are of frequent occurrence. Araldite-Epon.

X 3600

Figure 84. Four-day progesterone. A nucleus of a mucous cell is cut off from contact from the cytoplasm by the mucous granules (MUC). The indentation of the nuclear membrane in many cases follows the contour of the mucous granules (arrows). The fibrillary nature of the mucous content can clearly be seen here. Araldite-Epon.

X 23,430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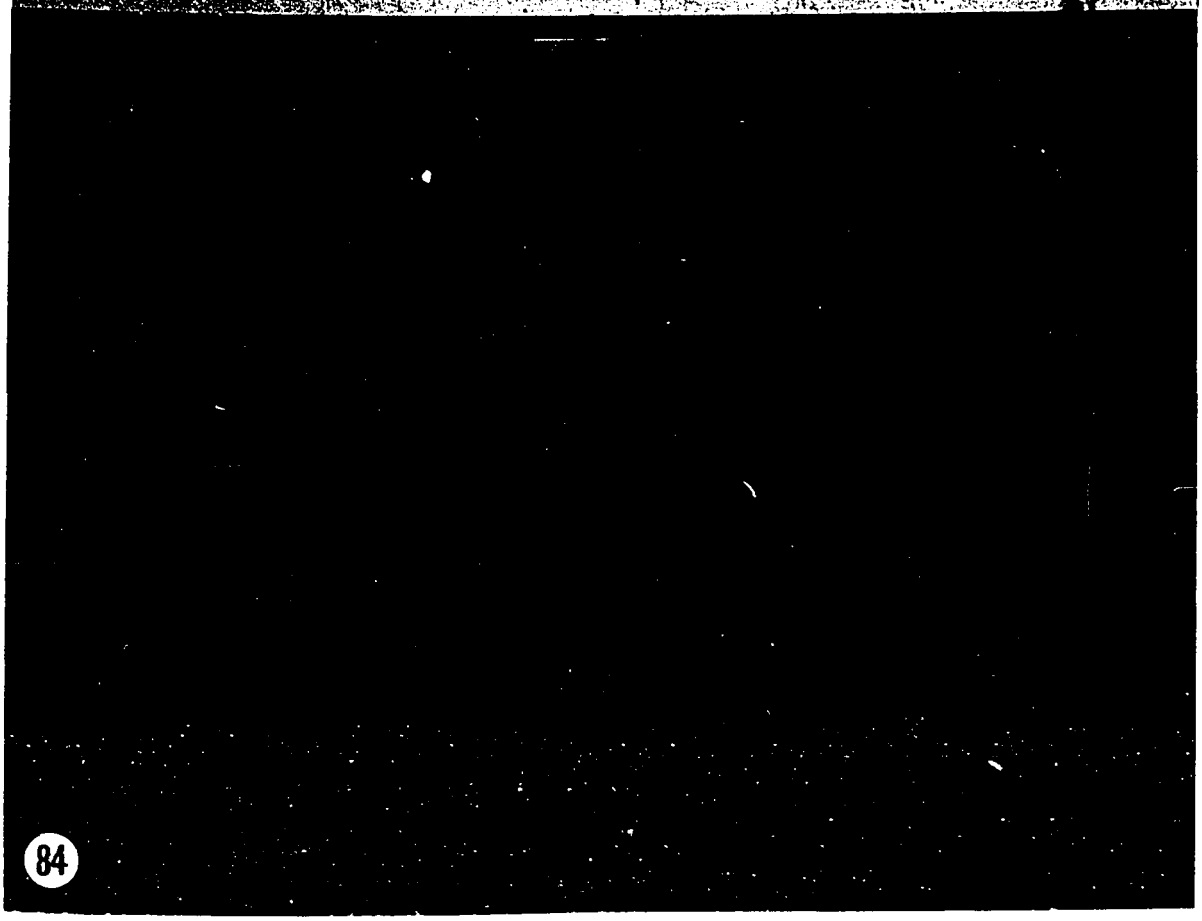
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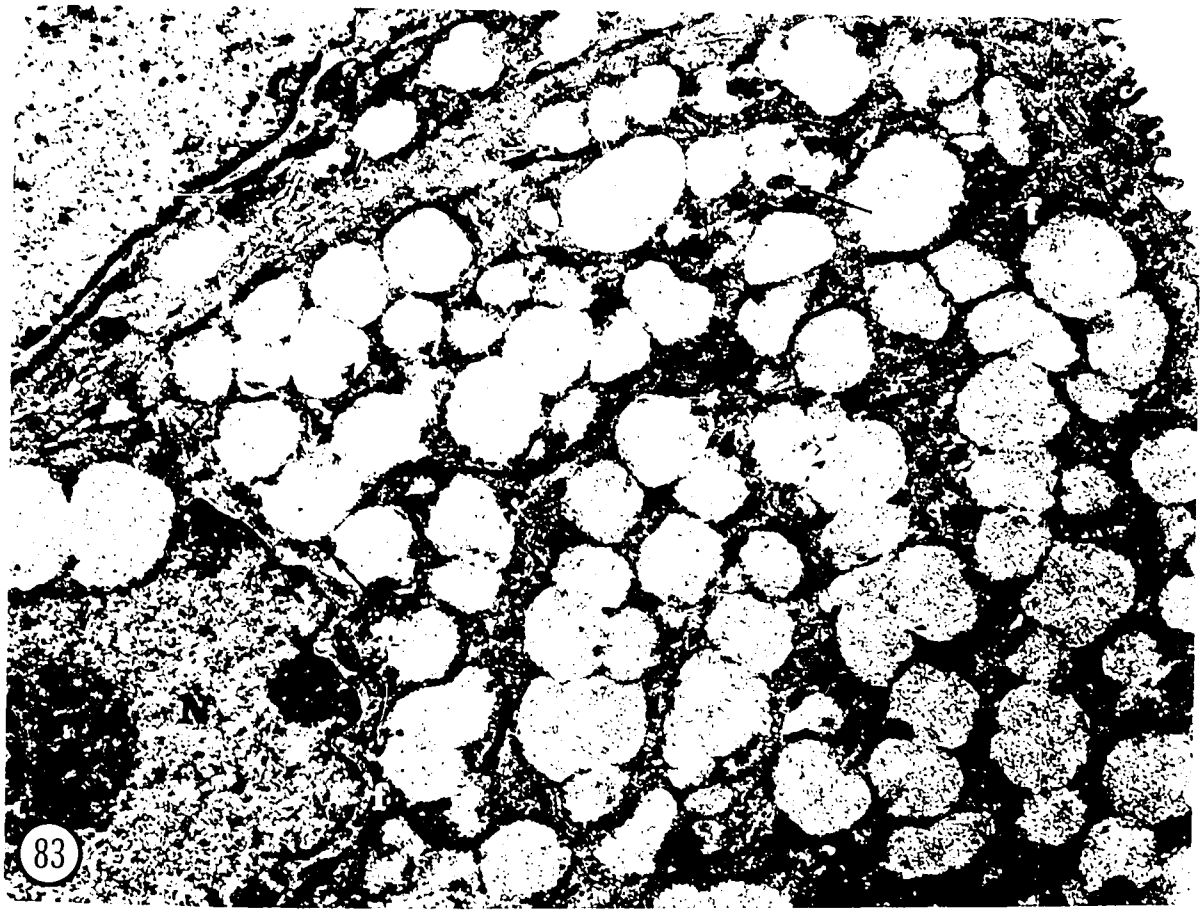

N
83

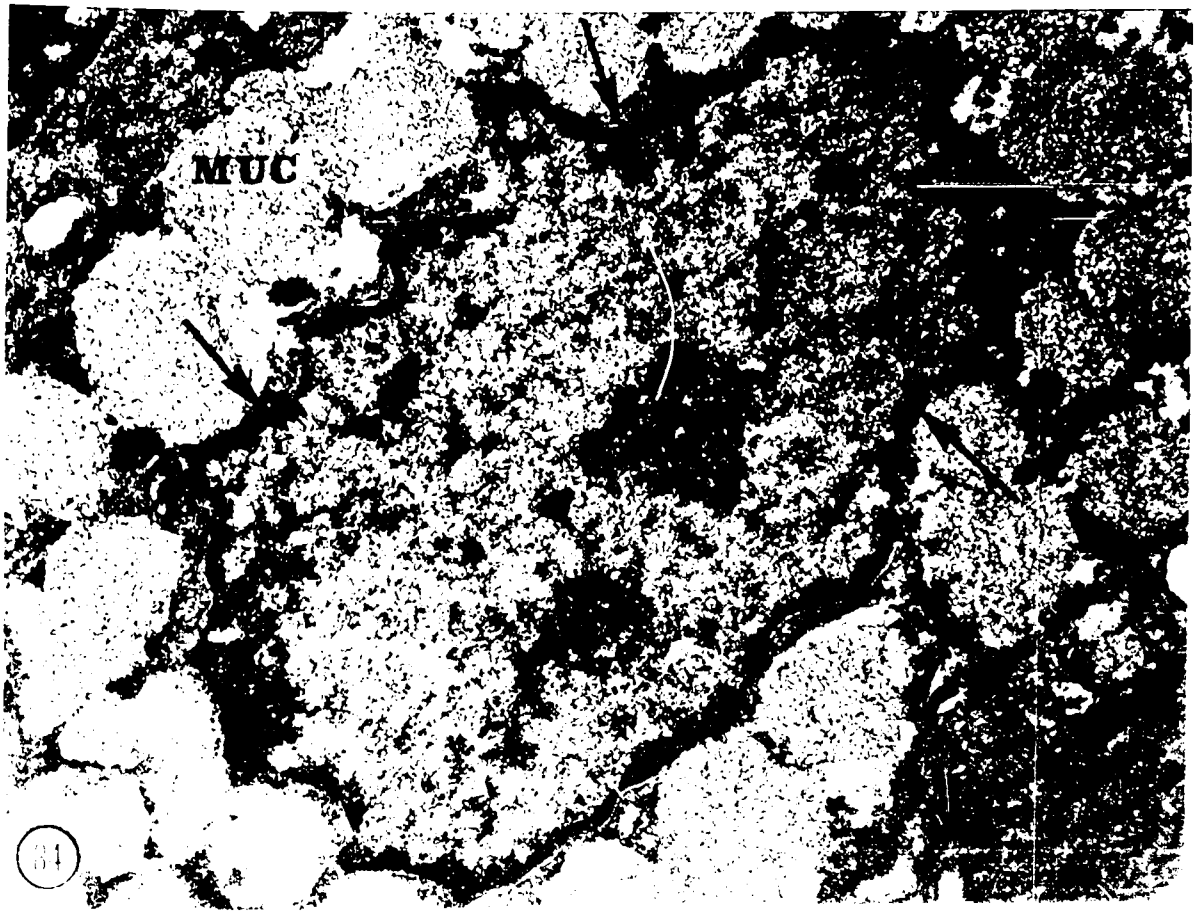
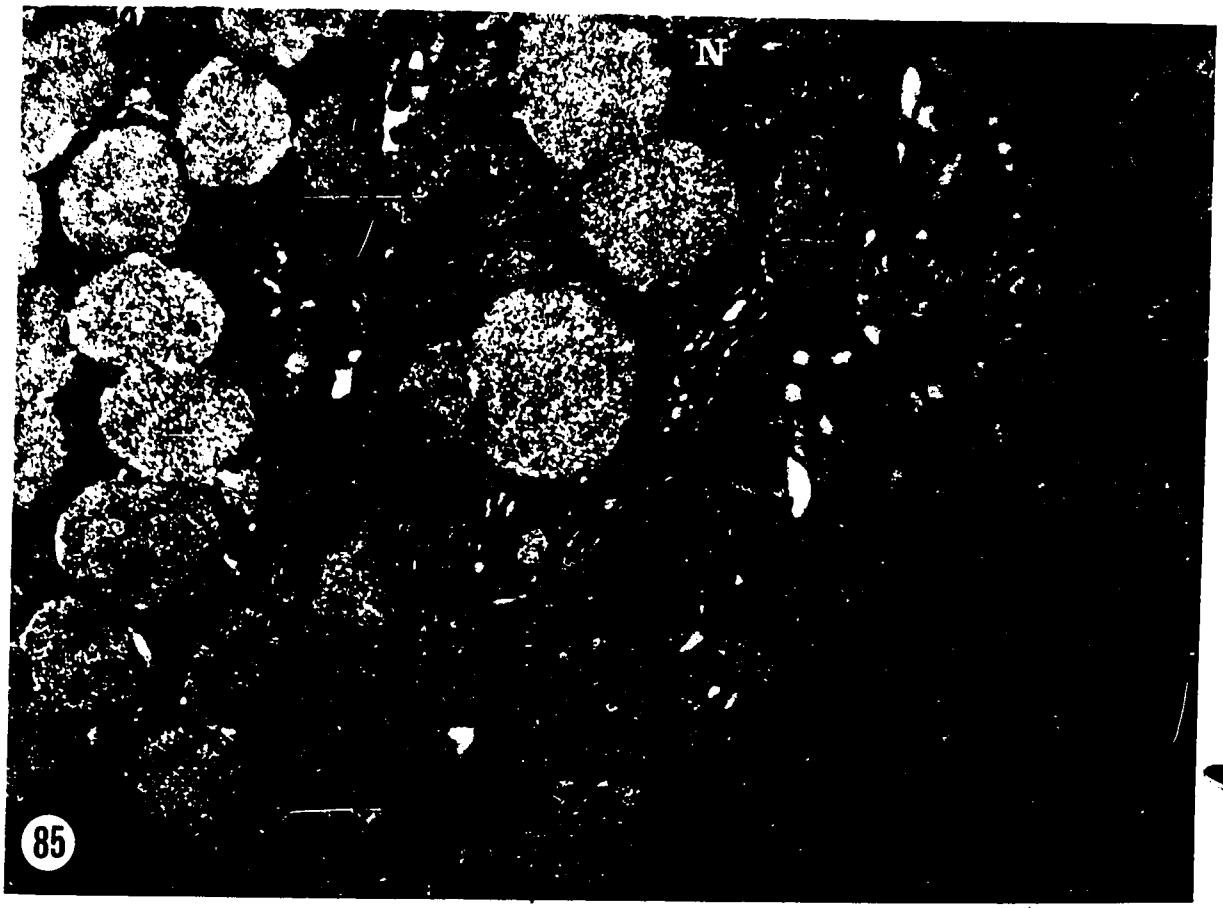

MUC
84

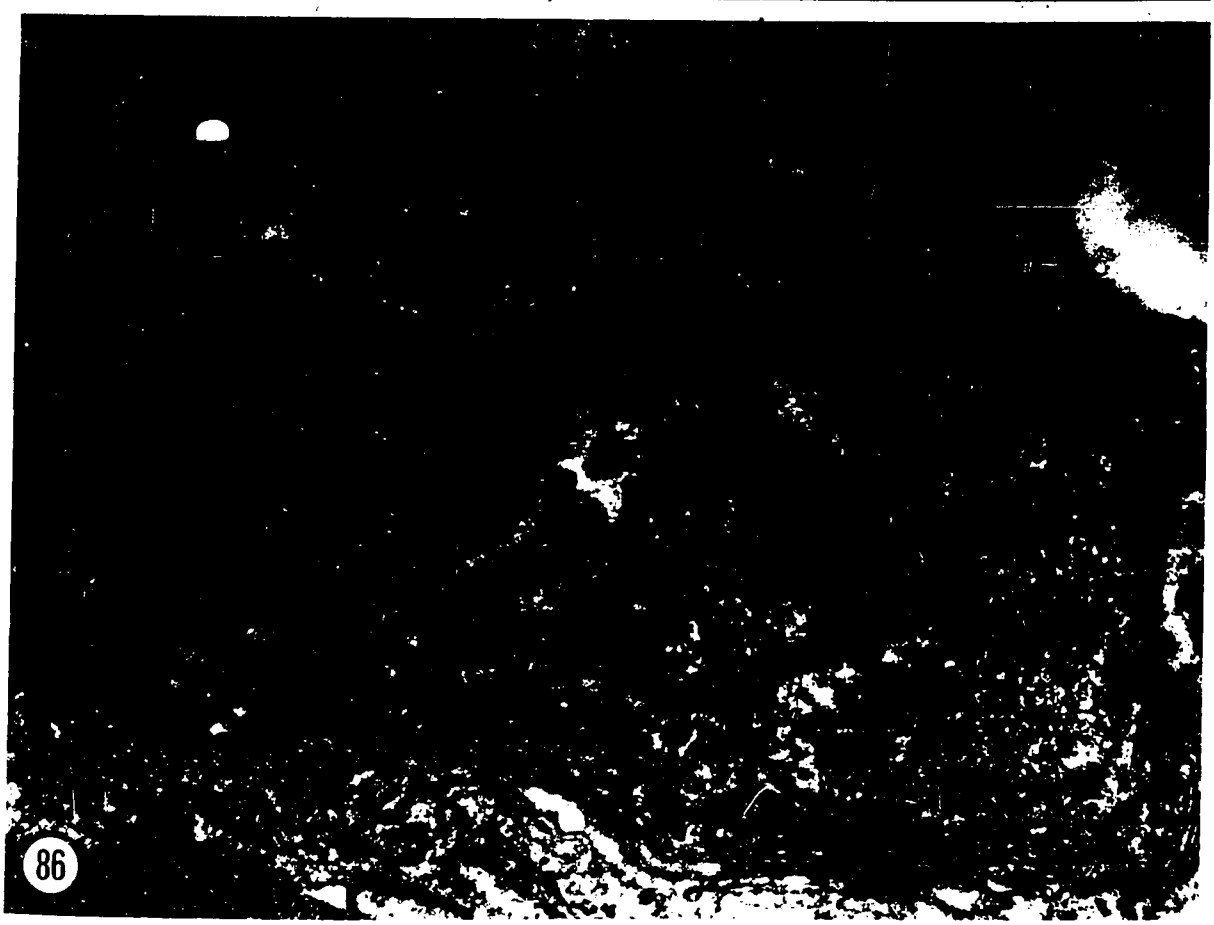
Figure 85. Four-day progesterone. A Golgi apparatus is shown to be located beneath the nucleus (N) of the mucous cell which is partially shown at the upper left of the picture. The nucleus at the bottom of the picture belongs to a basal cell. Mucous granules are closely associated with the Golgi membranes. Araldite-Epon.

X 23,430

Figure 86. Seven-day progesterone. High magnification of a Golgi of a mucous cell. Mucous granules, stacks of Golgi membrane, and a large number of small Golgi vesicles (v) are shown. A mucous (MUC) granule which is still connected to the Golgi membrane by a stalk (➡) can clearly be seen. Araldite-Epon.

X 57,500


N
85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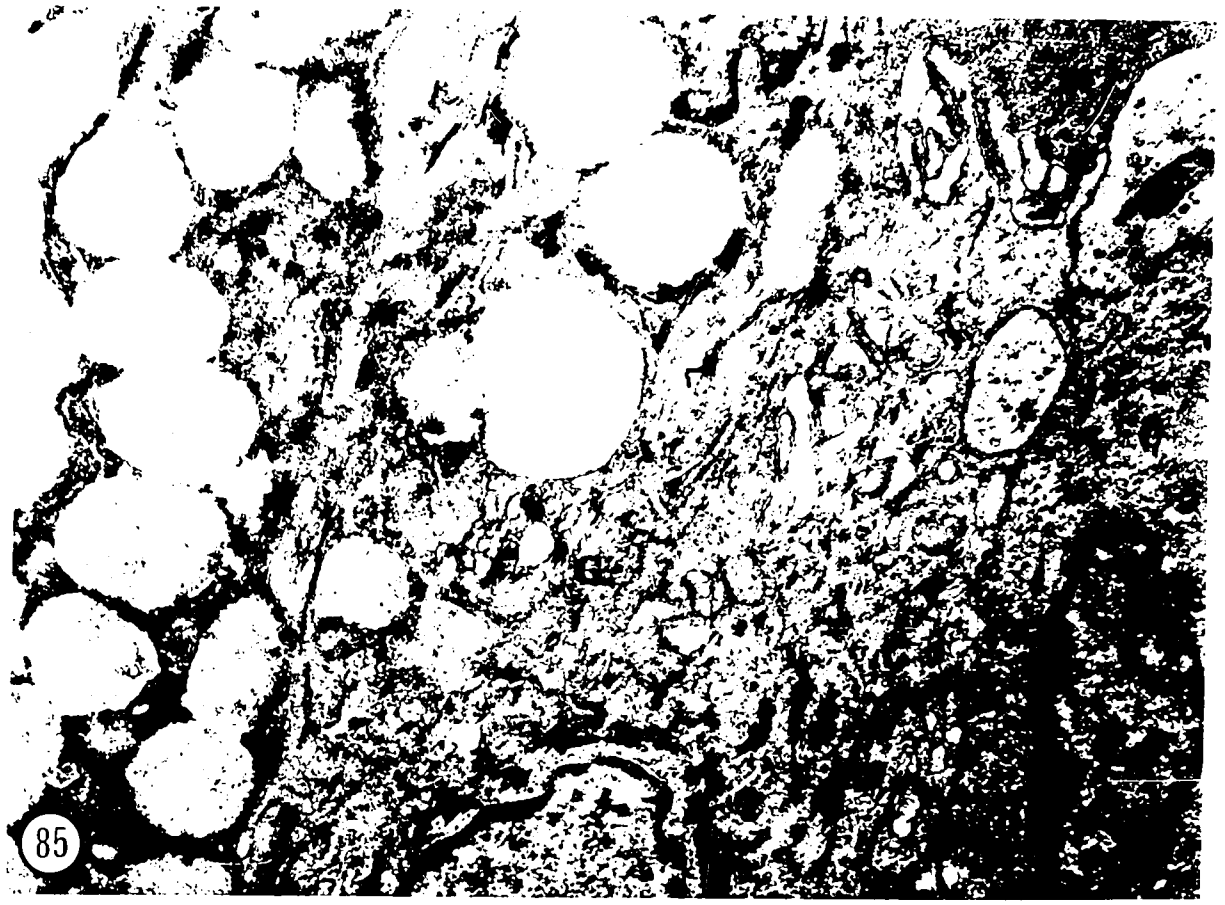


85

86

Continuity between the cisternae and the mucous granules of various size is shown in Figure 86. The granules at the Golgi zone appeared denser and had better defined limiting membranes. The membrane of the granules in other regions was mostly incomplete.

Due to the accumulation of large numbers of mucous granules, other organelles (mitochondria, RER) were crowded into the small amount of remaining space which was, therefore, rather dense. It is particularly worth noting that a considerable number of filaments (presumably keratin) were found in these mucous cells (Figs. 82, 87).

Mucous metaplasia in vaginal epithelium induced by progesterone, as in this case, was very uniform, and almost all the surface cells were affected, some, however, having more mucous granules than others.

The basal columnar layer was one or two cells thick (Fig. 88). The cytoplasm was dense and packed with ribosomes. The RER was scattered and keratin fibrils were present. The basal lamina was highly convoluted, and the cytoplasmic fingers of basal cells extending deep into the lamina propria (Fig. 88).

(e) Animals treated with estrogen and progesterone

When animals were treated with estrogen (0.2μg) and progesterone (1 mg.) simultaneously for four to eight days, the effects of each hormone were reflected in changes in the vaginal epithelium. These are shown in Figures 89-94.

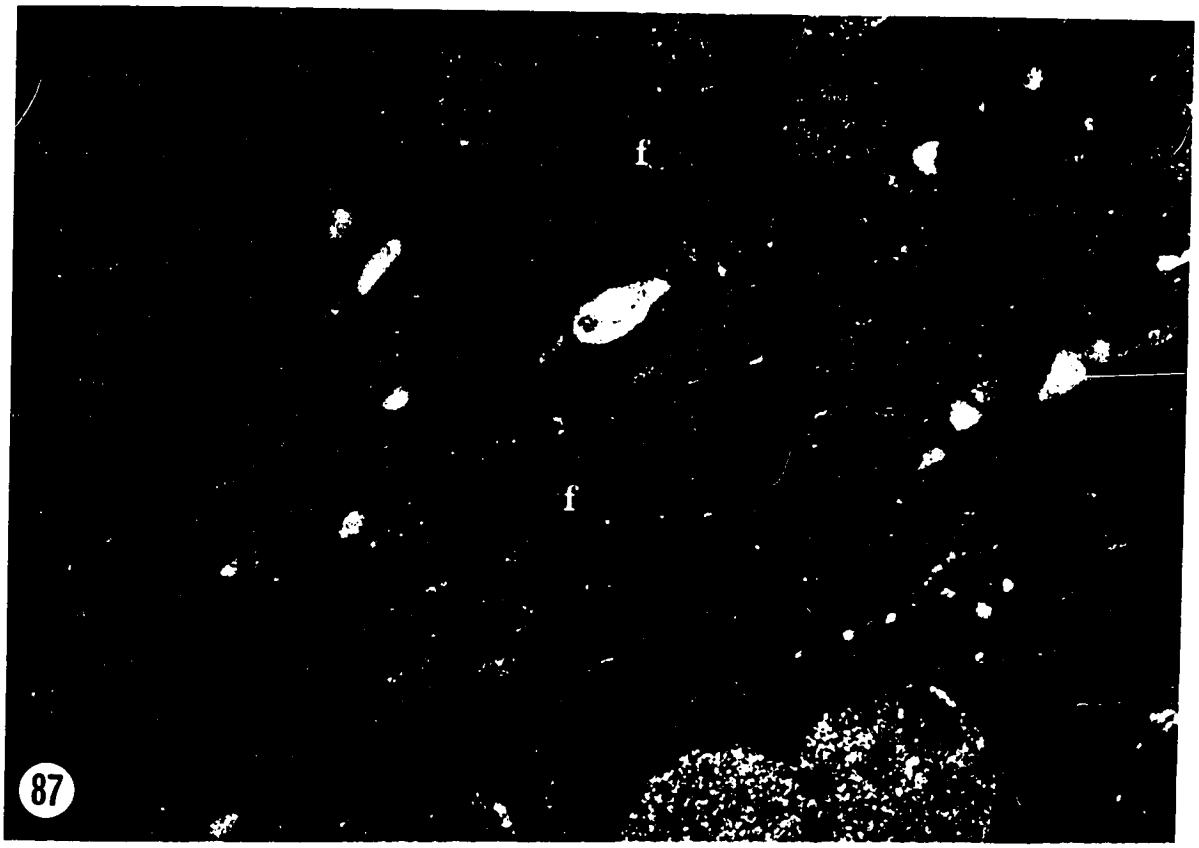
The epithelium was stratified squamous but without cornified layers (Figs. 89, 90). The basal and intermediate cells contained a large amount of keratin and well developed desmosomes. In addition, glycogen aggregates were seen. These cells bore a striking resemblance

Figure 87. Four-day progesterone. Portion of three adjacent mucous cells showing keratin fibrils (f) in the cytoplasm. Araldite-Epon.

X 8120

Fig. 88. Four-day progesterone. Basal cells are columnar in shape. The cytoplasm is dense and packed with ribosomes. Keratin fibrils are present in moderate amount in the cytoplasm. The basement membrane (BM) is highly convoluted, the cytoplasmic fingers extending deep into the lamina propria (LP). Araldite-Epon.

X 11,160



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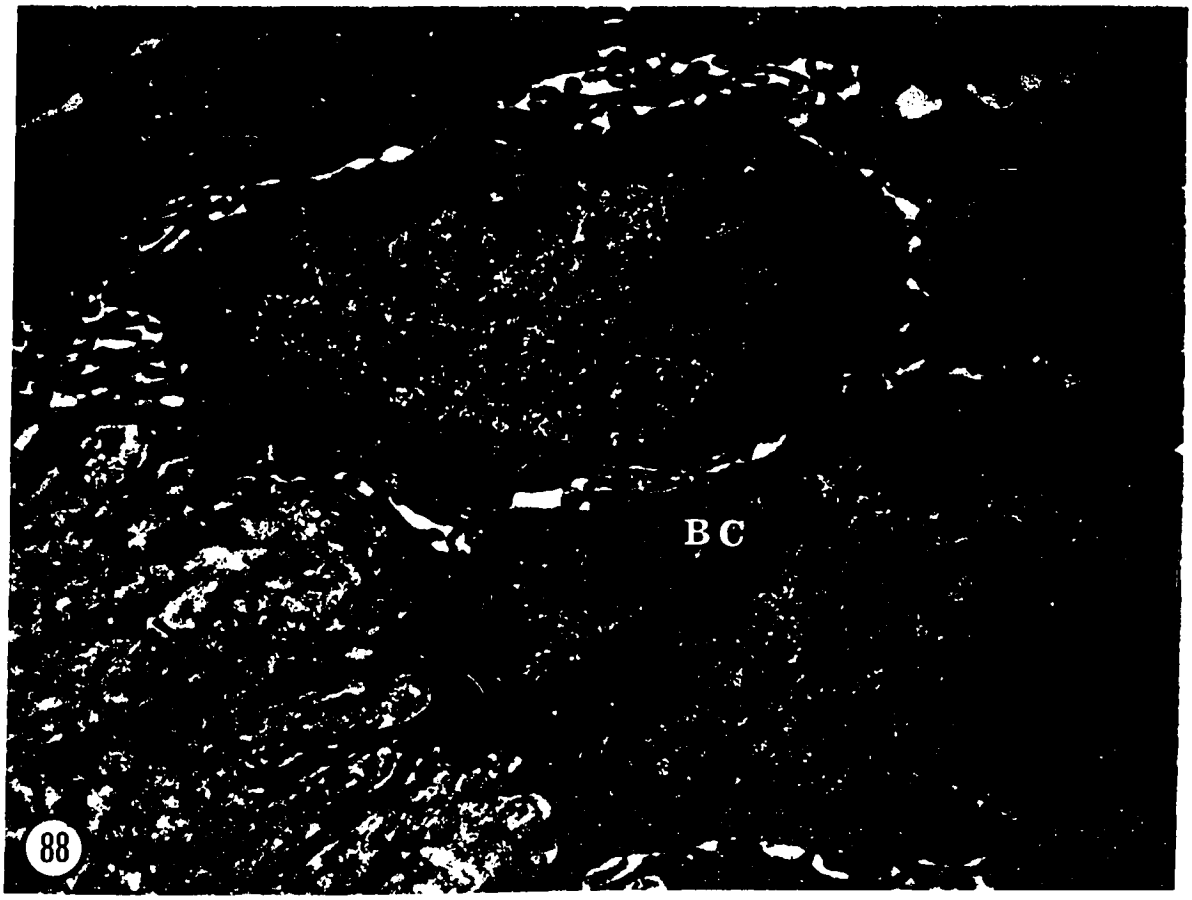

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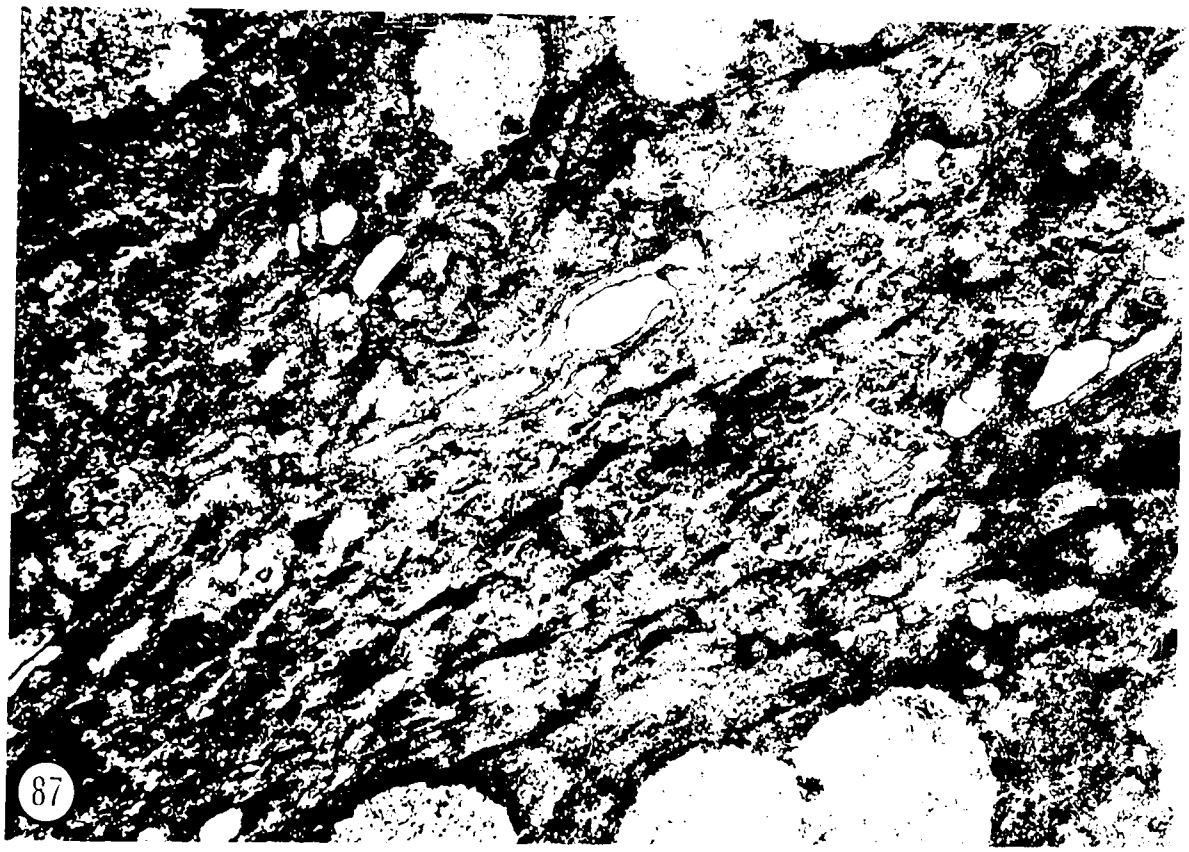
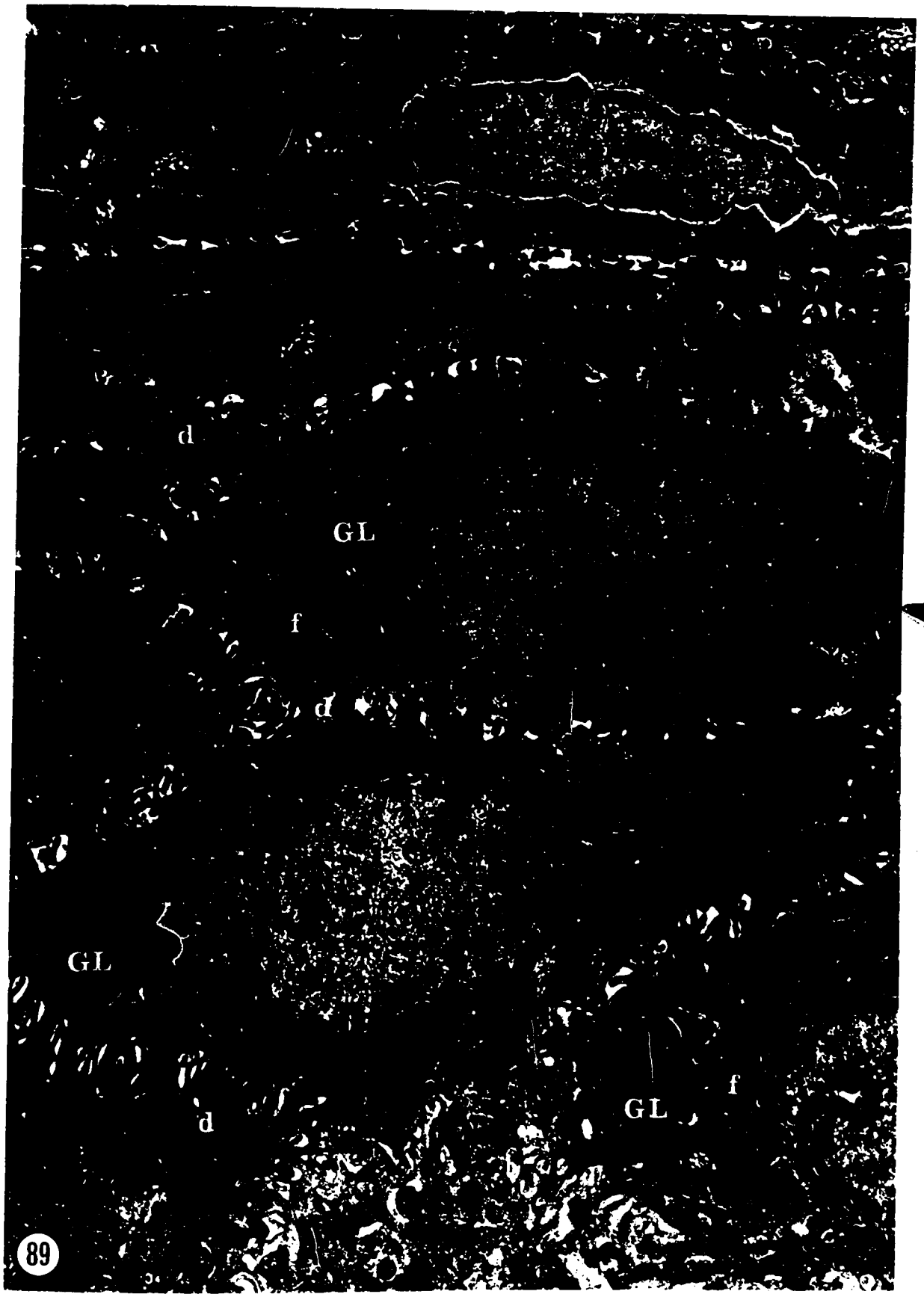

87


BM
LP

Figure 89. Six-day progesterone and estrogen. Typical spinous cells which contain large amounts of keratin fibrils (f) and well developed desmosomes (d) are shown in this picture. Glycogen (GL) aggregates are commonly seen in this epithelium. Durcupan.

X 10,500


d
GL
f
d
GL
d
GL
f
89

89

to those of the estrogen treated vaginal epithelium. Tight junctions were observed between these cells. However, the surface, squamous, non-cornified layers had entirely distinctive features (Figs. 90, 91). In addition to the keratin fibrils and glycogen aggregates, a large number of granules which were believed to be equivalent to mucous granules were observed (Figs. 90, 91). Some granules consisted of electron dense and light material (Fig. 91). However, most granules were much smaller than those induced by progesterone alone, were of much higher density (Figs. 90, 91), and were clearly bounded by a membrane. Their diameter ranged from 300 mμ to 500 mμ. Membrane-coating granules and KH granules, although prominent in the estrogen treated animals, were not observed following combined estrogen-progesterone treatment. The intercellular spaces of the squamous cells were larger than those of the basal and intermediate ones. Leucocytes were not infrequently found.

In some instances, single or clumps of columnar cells were found among the squamous cells even in the superficial layers (Figs. 92, 93). Keratin, RER, and other organelles were then as prominent as in the squamous cells described above. Mucous granules were of similar size and density, except that the number of "mixed" granules and light mucous granules had increased (Figs. 93, 94). The GA of these columnar cells might be multiple and was usually located in a supranuclear position. Some dense granules could be seen associated with it.

7. Cartilaginous and Bony Metaplasia in Regenerating Tendon

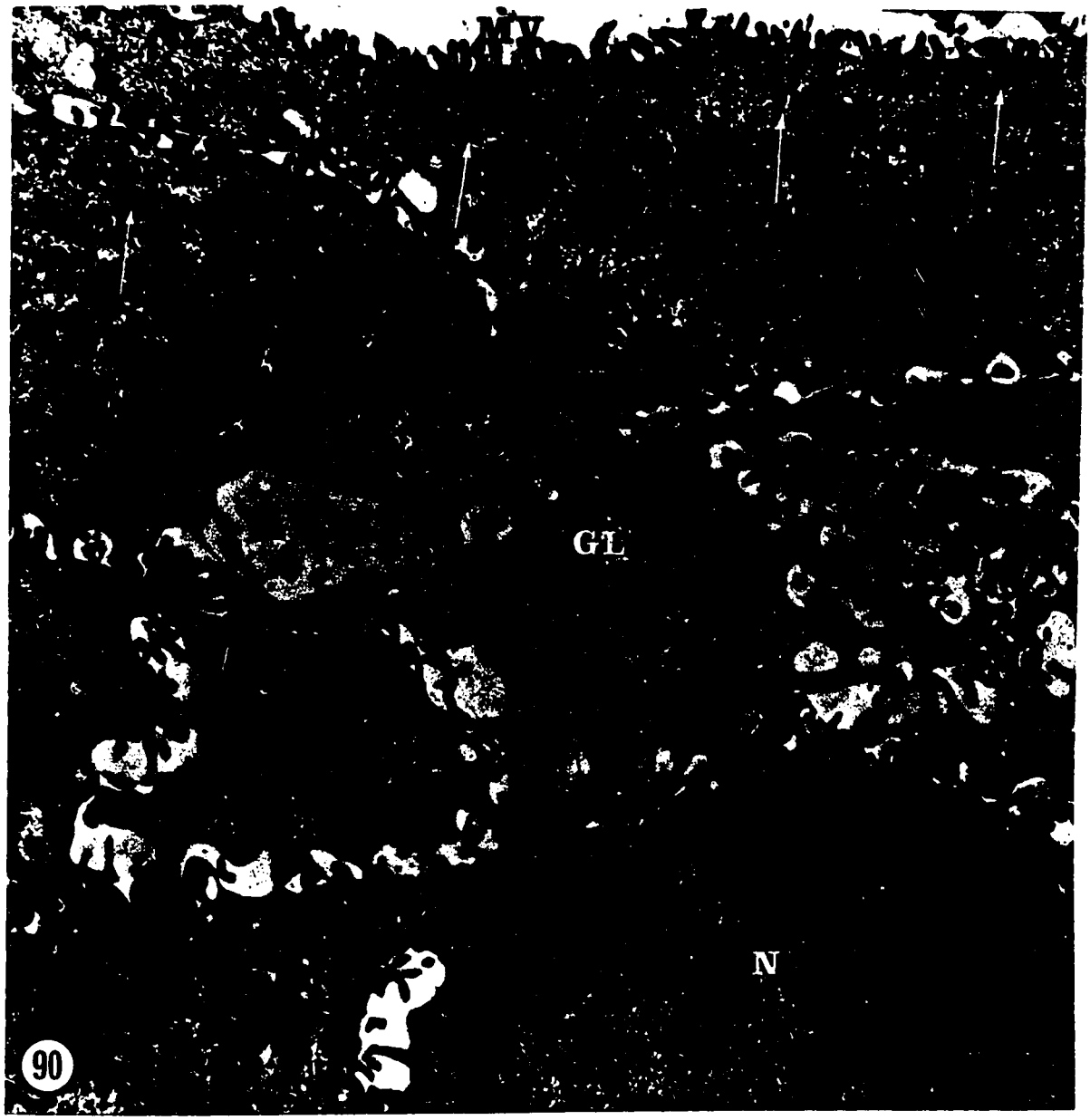
The regeneration of Achilles tendon in the rat has previously been reported both at the light (Buck, 1953) and electron microscopic

Figure 90. Eight-day progesterone and estrogen, showing a portion of the surface squamous non-cornified layers of vaginae epithelium. The intercellular spaces are wide, and microvilli (MV) are found both on the free edge and in the intercellular spaces. Glycogen (GL) aggregates are also present. A large number of granules (arrows) which are twice or three times the size of MCG are believed to be equivalent to the mucous granules in progesterone treated animals. Aradite-Epon.

X 12,800

Fig. 91. At higher magnification these granules are seen to be bounded by a membrane. The dense matrix often does not fill the granule completely, leaving an electron light area. Araldite-Epon.

X 59,200



90

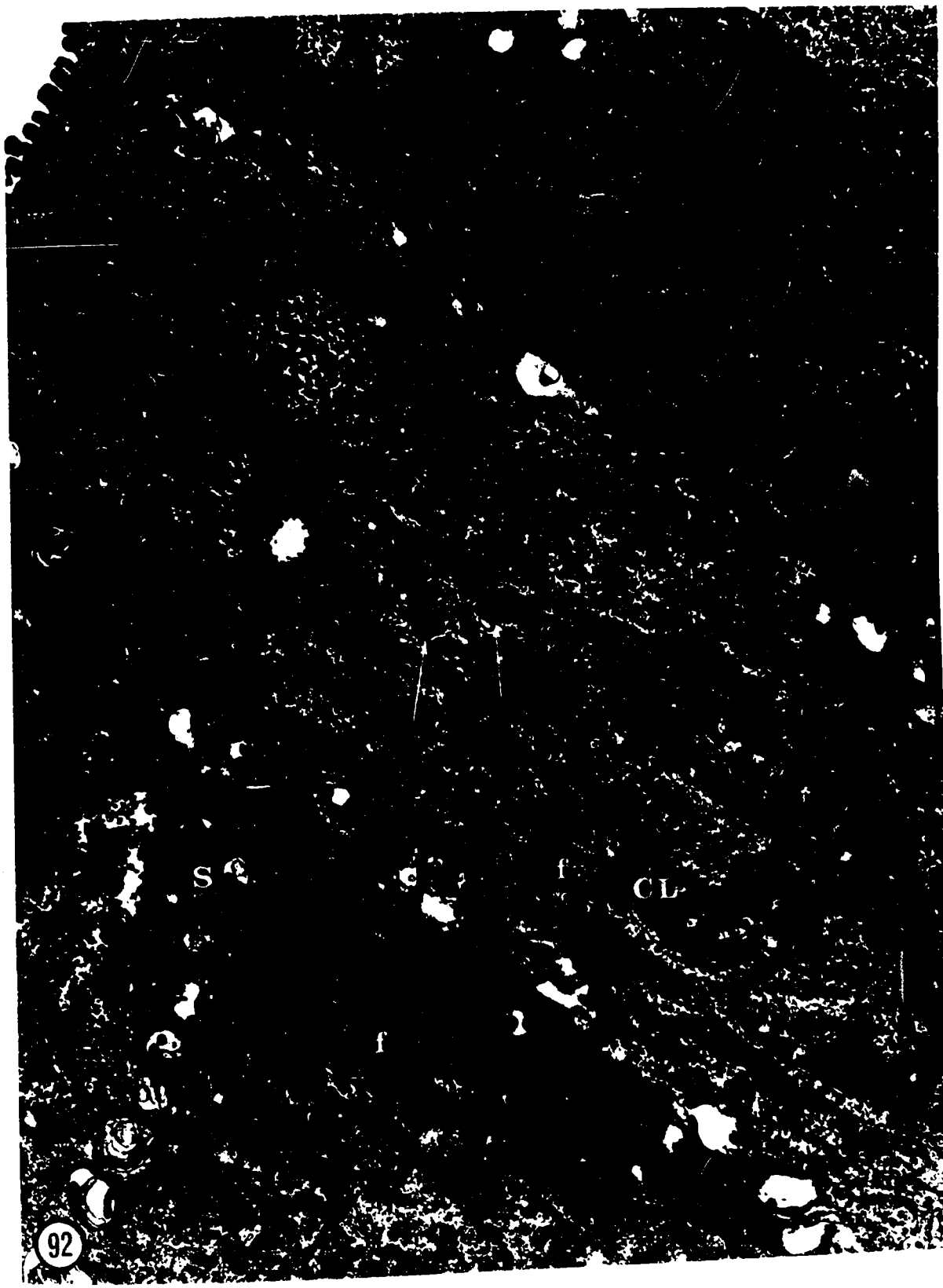
91

90

91

Figure 92. Eight-day progesterone and estrogen. A columnar cell (CL) is seen located among the superficial squamous cells. It has a deeply indented nucleus. The RER is prominent and large numbers of keratin fibrils (f) are present in the cytoplasm. Small mucous granules can be seen here and there in the cytoplasm, two of which are indicated by arrows. Glycogen (GL) aggregates are also seen both in columnar and adjacent squamous cells (S).

X 14,400


S
f
CL
f
92

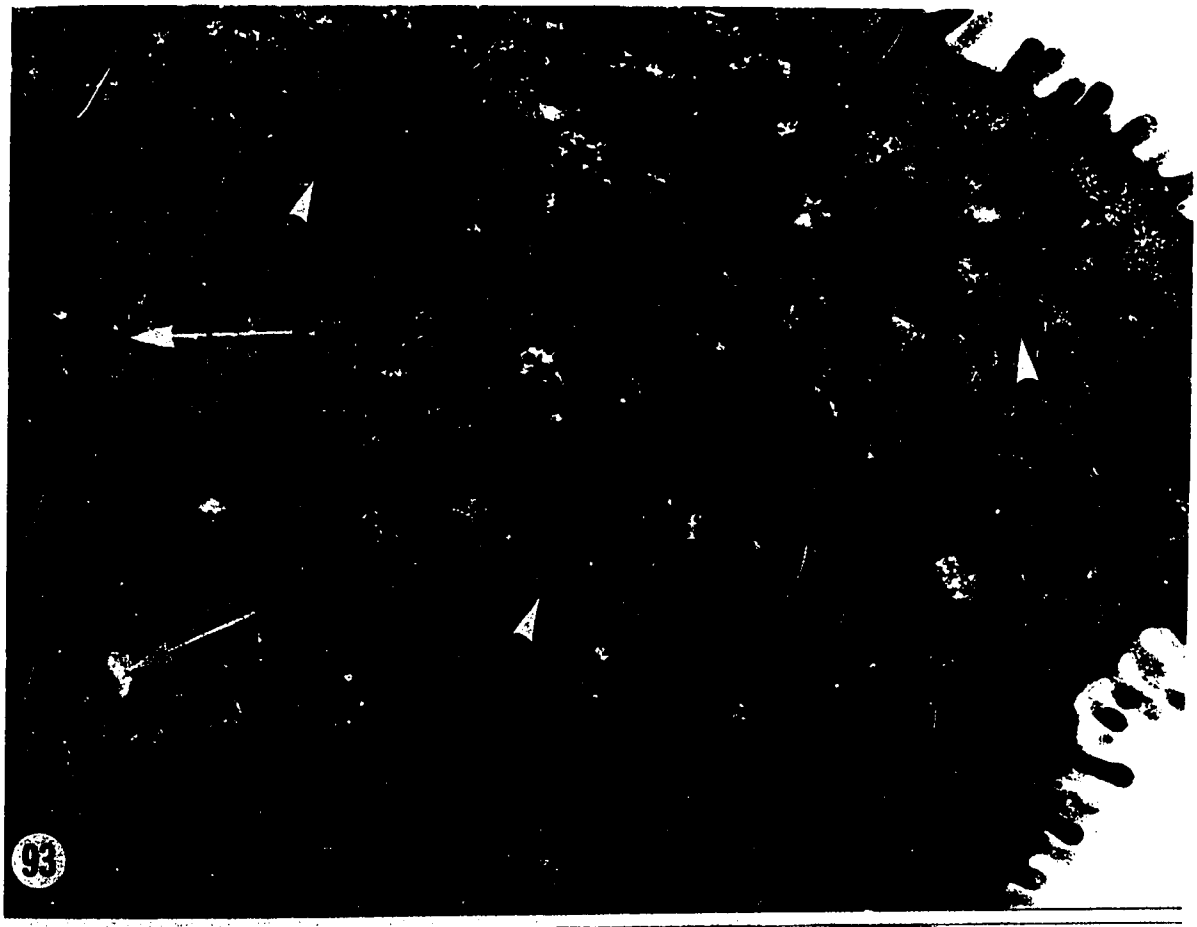

GL
92

Figure 93. Eight-day progesterone and estrogen, showing the apical cytoplasm of a columnar cell. A large number of granules (➤) is present in the cytoplasm. They are comparable to those found in the squamous cells under similar condition. In some of the larger ones (arrows) electron light regions can be seen in the granules (mixed granules). Keratin fibrils (f) are prominent in the cytoplasm. Araldite-Epon.

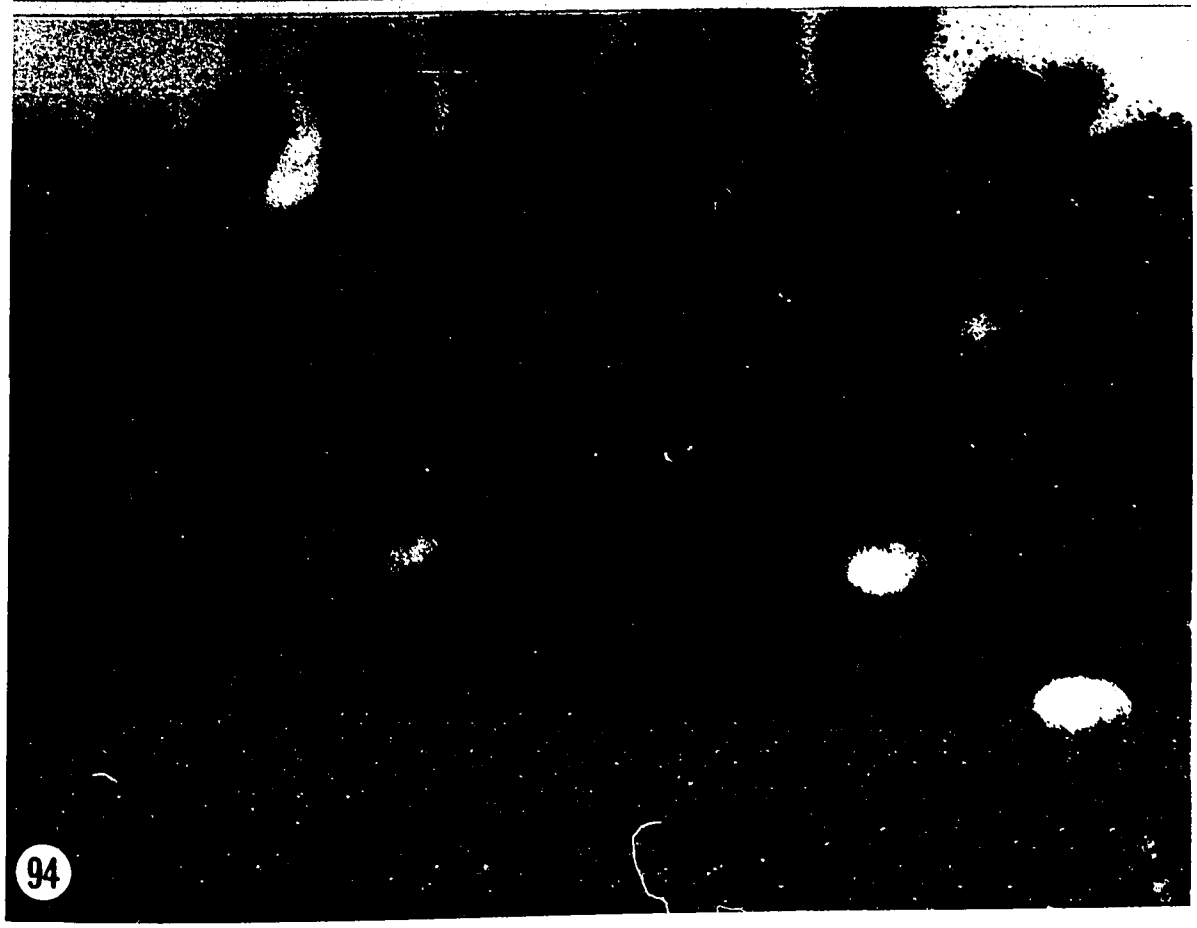
X 12,680

Figure 94. Eight-day progesterone and estrogen. At high magnification, the granules are seen to be bounded by unit membrane. Most of the granules are filled or partially filled by electron dense material. Araldite-Epon.

X 87,500



93



94

93

(Fernando and Movat, 1963) level, the detail of which will not be given here. The former also reported the observation of cartilaginous and bony metaplasia in the regenerating tendon. This observation formed the basis of present, more systematic, investigation.

(a) Light microscopic observation.

From animals which were sacrificed at one, two, three, four or five weeks after tenotomy, the regenerating tendons were serially sectioned and by staining with toluidine blue for metachromasia, the number of sites of metaplastic change was determined. Fifteen sections from each tendon were randomly selected and the number of metaplastic sites was counted. They were classified as pre-cartilaginous, cartilaginous, calcified cartilage and bony stages. The pre-cartilaginous stage is characterized by areas showing intense metachromasia, having with cells considerably less spindle-shaped than those of the rest of the regenerating tissue, but lacking definite lacunae. The results are summerized in Table 1 and Chart 1.

No metaplasia was observed in one-week regenerating tendon, although quite strong metachromasia was seen. However, in specimens obtained only three days later, several pre-cartilaginous sites were found. The cells at these regions were randomly arranged in contrast to the regularly arranged fibroblasts in the non-metaplastic regions. The matrix was highly metachromatic, although typical cartilaginous features were not seen at this stage (Fig. 95). As indicated in Chart 1, the number of pre-cartilaginous sites reached its peak in the second or third weeks and slightly decreased in the fourth and fifth weeks (Chart 1)

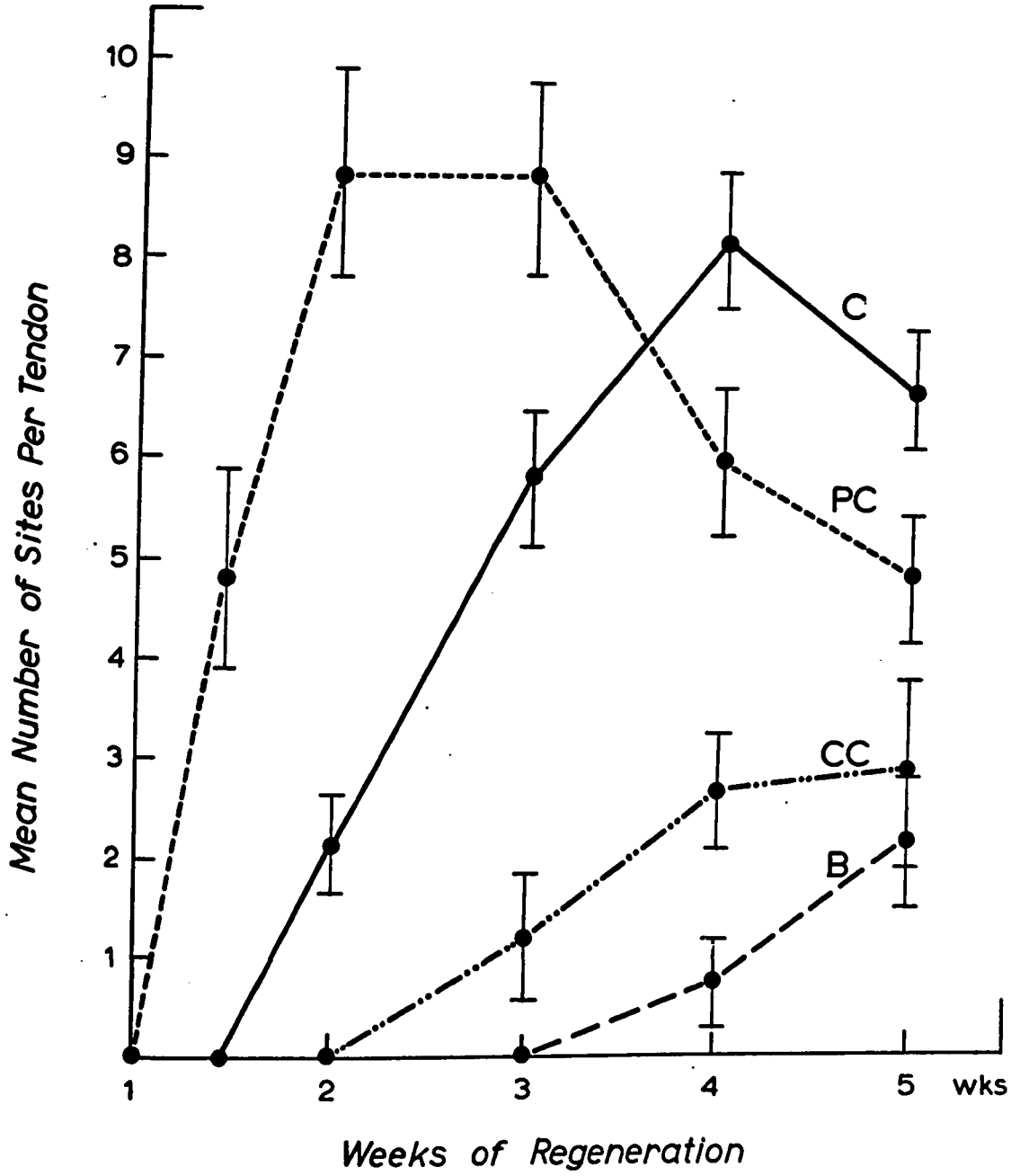
Age of Regeneration	No. of Animals	PC		C		CC		B	
		M	SD	M	SD	M	SD	M	SD
7-day	7								
10 "	5	4.8	±1.0						
14 "	10	8.8	±1.1	2.1	±0.5				
21 "	6	8.8	±0.9	5.8	±0.7	1.2	±0.6		
28 "	6	6.0	±0.7	8.2	±0.8	2.7	±0.7	0.7	±0.4
35 "	6	4.8	±0.6	6.7	±0.7	2.8	±0.9	2.2	±0.8
Total Animals	40								

Table 1. Mean number of metaplastic sites in regenerating tendon of the control animals (sites per tendon).

PC, pre-cartilaginous; C, cartilaginous; CC, calcified cartilage; B, bone

Chart 1. Graph showing the development of metaplastic sites in regenerating Achilles tendon of the rat in relation to duration of regeneration.

PC, pre-cartilaginous; C, Cartilaginous; CC, Calcified cartilage; B, Bone. Bars indicate standard error of mean.


Mean Number of Sites Per Tendon
10
9
8
7
6
5
4
3
2
1
C
PC
CC
B
1
2
3
4
5
wks
Weeks of Regeneration

A few sites of metaplastic cartilage were observed in the second week, increased dramatically in third week (Fig. 96, Table 1), and reached a peak at four weeks, by which time the number of PC sites had decreased (Chart 1). Calcification first occurred at the centre of metaplastic cartilage at the third week and the number of sites increased gradually (Fig. 97). Bony metaplasia was observed in the fourth and fifth weeks.

In general there were more metaplastic sites in the regenerating tendon at the point of junction with the old stumps, than in the centre of the regeneration region, or in the old stumps.

A few animals were allowed to regenerate for six months and X-ray pictures of the tendon were then taken. Bony islands were invariably found in the regenerated tendon (Fig. 98).

It should be noted that the absolute number of metaplastic sites is higher than presented here because any section would pass through many, but not all, the metaplastic sites.

(b) Transplantation of regenerating tendon.

Regenerating tendon including the old stumps of ages one, two, three, four or five weeks were auto-grafted to the subcutaneous tissue of the back for one, two, three or four weeks. Animals were sacrificed at appropriate times and the grafted tendons were removed and processed for light microscopy.

The results are summarized in Table 2 and Chart 2.

Figure 95. Three-week regenerating tendon. Light micrograph showing pre-cartilaginous stage of metaplasia. Toluidine blue.

X 400

Figure 96. Four-week regenerating tendon. A light micrograph showing metaplastic cartilage. Toluidine blue.

X 400

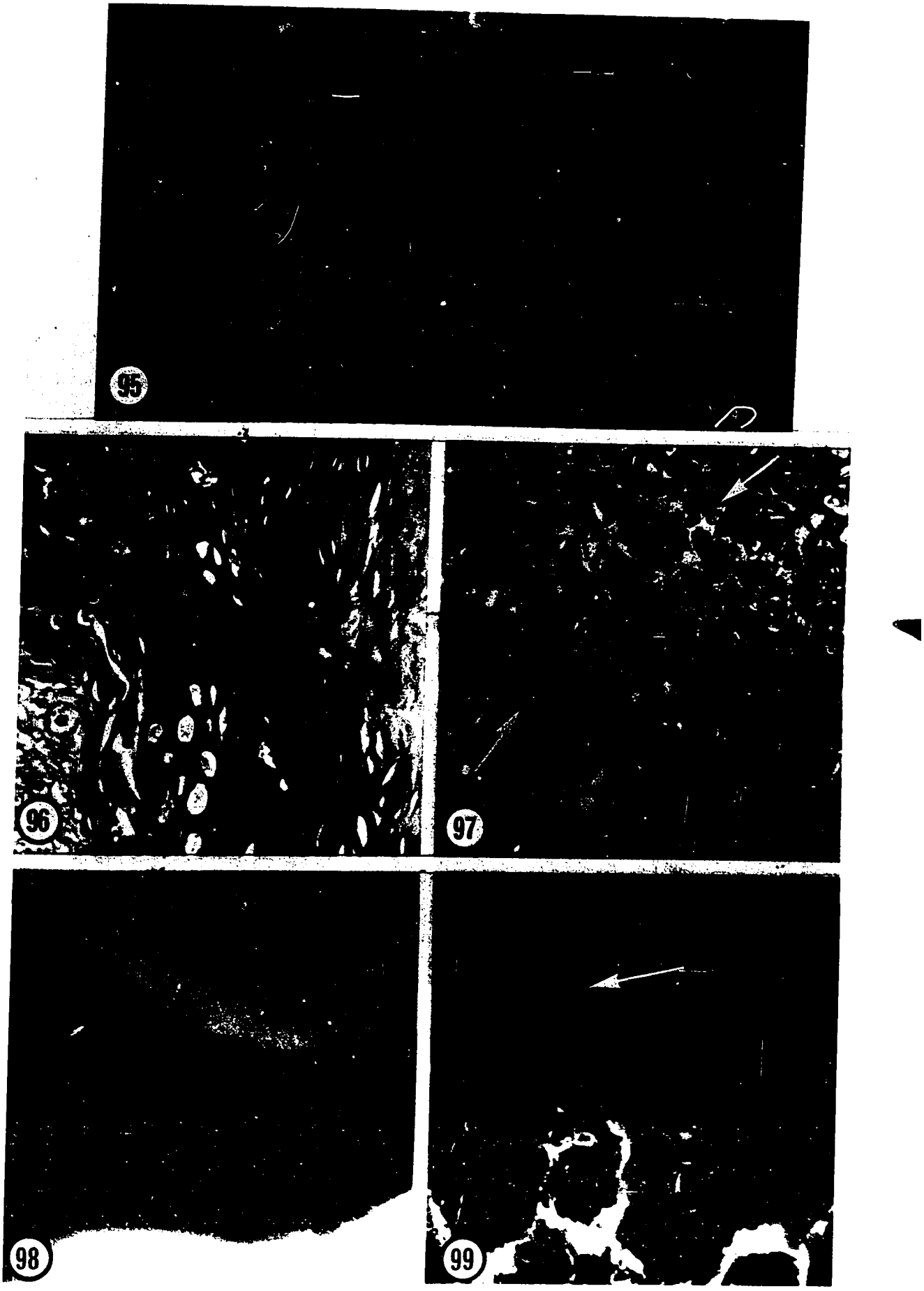
Figure 97. Four-week regenerating tendon. A region of calcifying cartilage. Limits of calcification are marked by arrows. Toluidine blue.

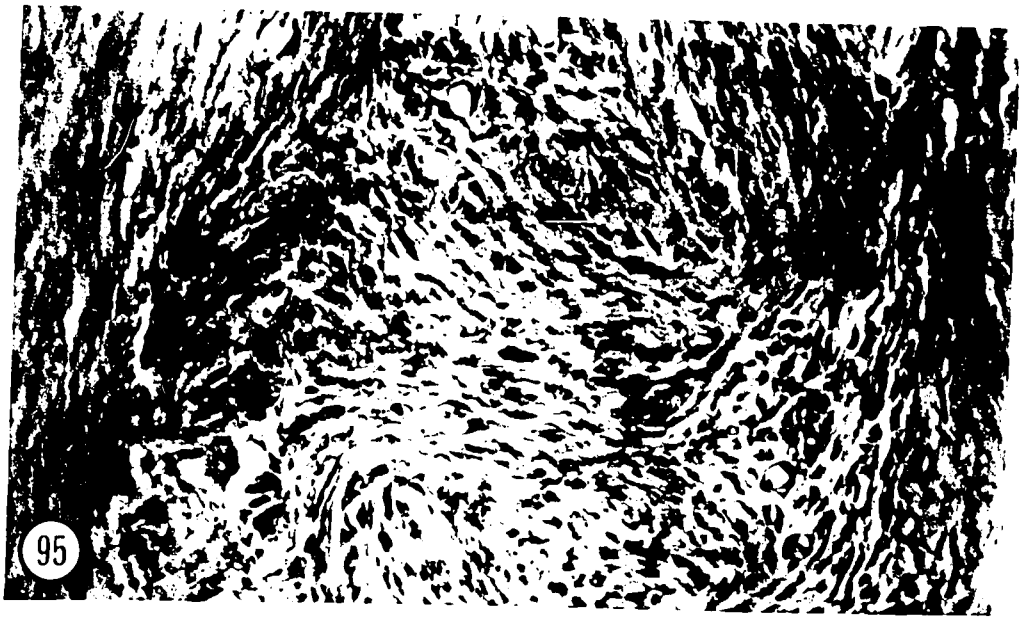
X 400

Figure 98. An X-ray picture of six-month regenerating tendon. A big metaplastic bone island is indicated by an arrow.

Figure 99. Three-week regenerating tendon, four-week transplantation. A piece of bone is shown. A row of osteoblasts is indicated by an arrow.

X 450


95
96
97
98
99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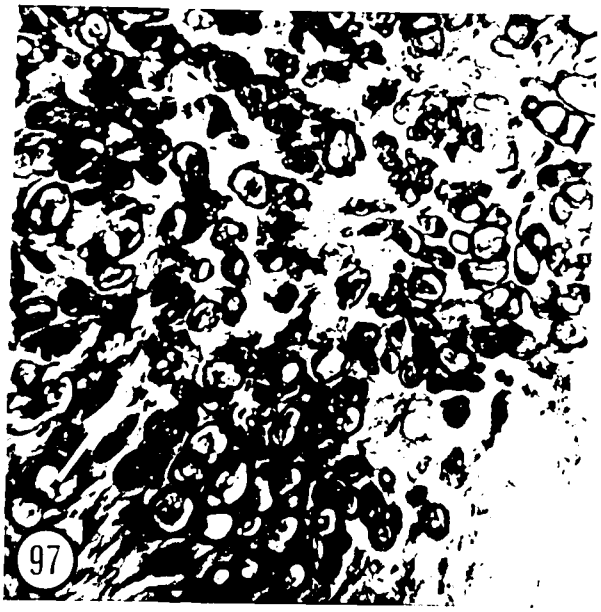

97

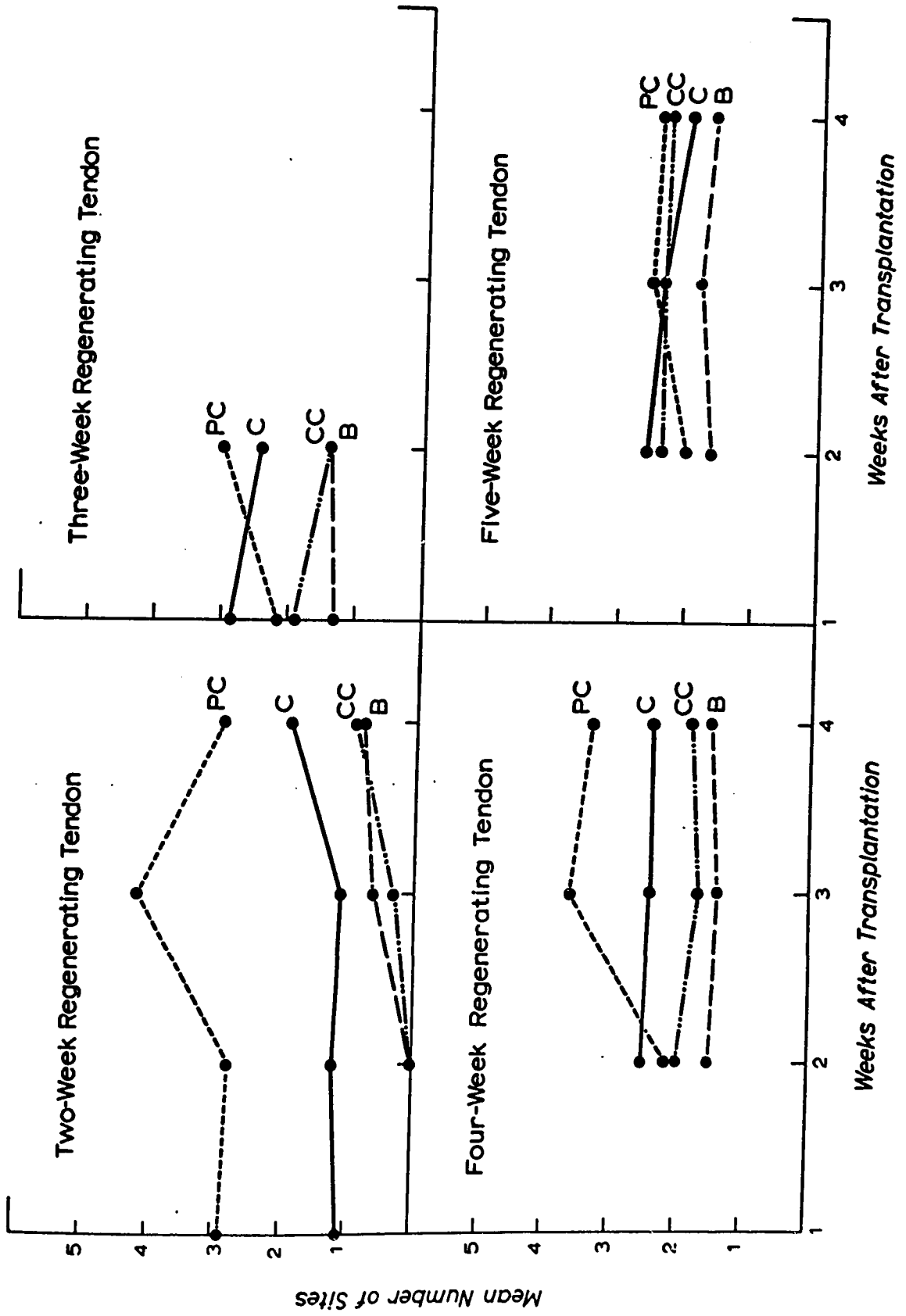

98


99

Day of Regeneration	Day after Transplantation	Number of Animals	PC		C		CC		B	
			M	SD	M	SD	M	SD	M	SD
7	7	7	-	-	-	-	-	-	-	-
14	7	9	2.9	±0.8	1.1	±0.6				
14	14	10	2.8	±0.8	1.2	±0.6				
14	21	12	4.2	±1.6	1.1	±0.9	0.3	±0.4	0.6	±0.6
14	28	14	2.8	±1.1	1.8	±0.8	0.9	±0.7	0.8	±0.7
21	7	9	2.1	±0.4	2.9	±0.4	1.9	±0.4	1.3	±0.6
21	14	9	3.0	±0.7	2.4	±0.6	1.4	±0.6	1.4	±0.5
28	14	8	2.1	±0.4	2.5	±0.5	2.0	±0.6	1.5	±0.5
28	21	8	3.6	±0.6	2.4	±0.5	1.7	±0.5	1.4	±0.6
28	28	10	3.3	±0.6	2.4	±0.5	1.8	±0.8	1.5	±0.6
35	14	5	2.0	±0.4	2.6	±0.5	2.4	±0.5	1.6	±0.5
35	21	8	2.5	±0.6	2.4	±0.5	2.4	±0.7	1.8	±0.7
35	28	7	2.4	±0.8	2.0	±0.3	2.3	±0.6	1.6	±0.4
Total Animals		116								

Table 2. Mean metaplastic sites in regenerating tendon after transplantation (sites per graft).

Chart 2. Relationship between number of sites of metaplastic tissue in grafts of regenerating tendon implanted to subcutis at various times after transplantation and following various periods of regeneration.


Two-Week Regenerating Tendon
Three-Week Regenerating Tendon
Four-Week Regenerating Tendon
Five-Week Regenerating Tendon
PC
C
CC
B
Mean Number of Sites
Weeks After Transplantation
1
2
3
4
5

In general, the size of the tendons after two weeks of explantation was reduced to half the initial size, and the metachromasia of the tendons was weaker.

There was no metaplastic site observed in grafts of one week regenerating tendon (Table 2) . In tendons transplanted after two or more weeks of regeneration, cartilage and bone islands were invariably present (Fig. 99; Table 2; Chart 2). However, the number of these was smaller than that in the control animals and there was little change seen in the number of metaplastic sites in all the transplanted tendon (Table 2; Chart 2).

(c) Electron microscopic observations

The details of fibroblasts and fibrillogenesis in the regenerating tendon will not be presented here as they are similar to those of other connective tissues and have been extensively studied (Movat and Fernando, 1962; Fernando and Movat, 1963; Fitton-Jackson, 1968; Ross, 1968; Haust and More, 1967). However, a brief description wil be given.

(i) Fibroblast

The fibroblast in the regenerating tendon was elongated or stellate in shape with numerous cytoplasmic projections on the surface. In accordance with the light microscopic observation, most of the cells were arranged parallel to the direction of tensile force. The cells were usually large and the cytoplasm was abundant. The nucleus was

also elongated in shape and quite often located at one end of the cell. It had one, or sometimes more, nucleoli. The cytoplasm was characterized by the presence of a highly developed GA close to the nucleus (Fig. 100). However, the GA in the fibroblast might be multiple and not necessarily confined to a particular region of the cell. It consisted of flattened lamellae and vesicles. Many of the larger vesicles contained fibrillary material. Often there was a distinct "halo" between the membrane and fibrillary material (Figs. 100, 101). Occasionally, fibrils bearing the characteristic banding of collagen were also observed (Fig. 102). Their location and frequency made it unlikely that this appearance was due to the sectioning of pockets of invaginated extracellular collagen fibrils. A similar finding has recently been reported in various types of osteogenic cells of fracture callus in the rat (Göthlin and Ericsson, 1970).

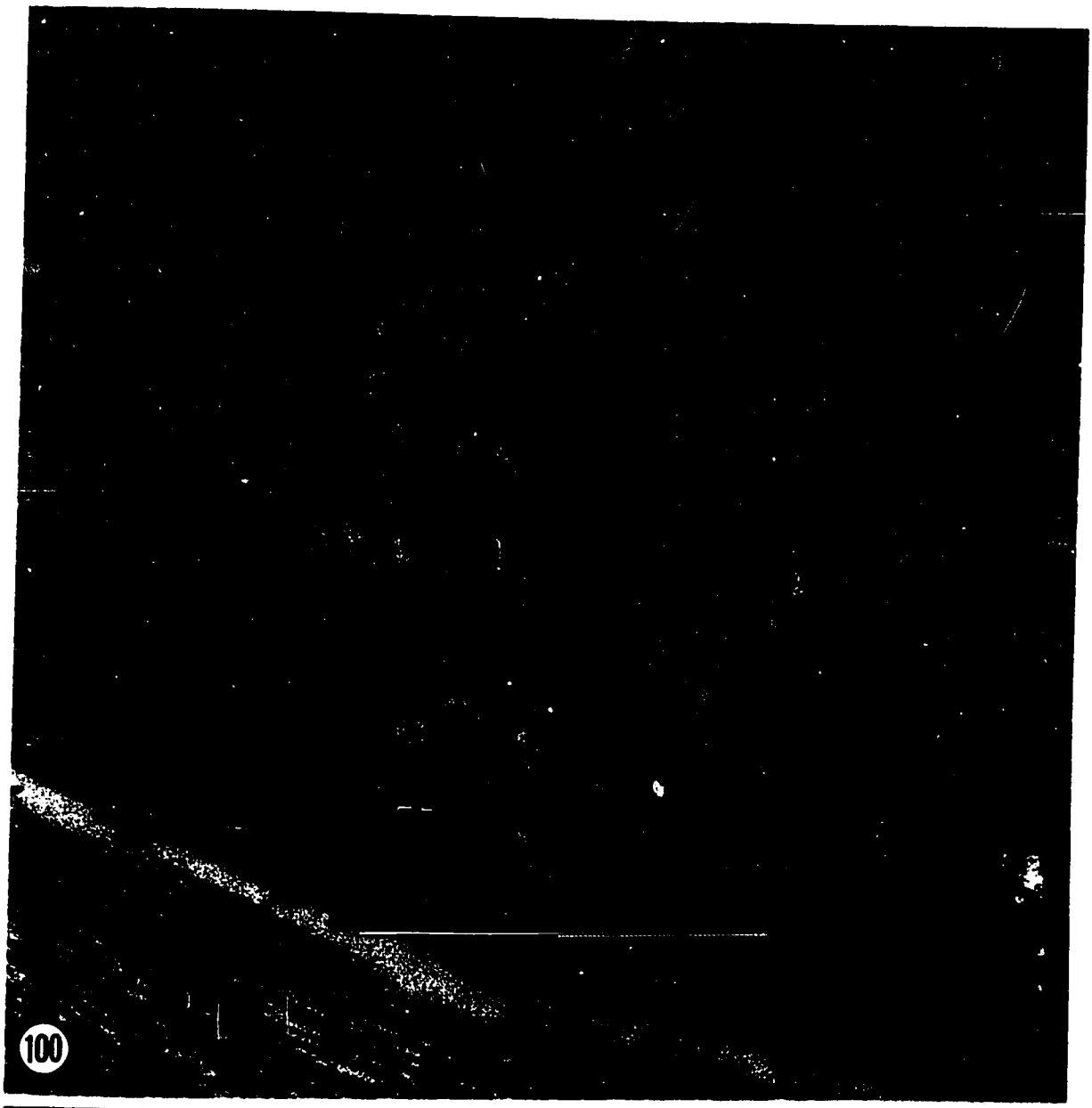
The RER of the fibroblast was very extensive, as expected in cells that are active in the synthesis of protein for transport (Porter, 1961). In some cells the cisternae of the RER were slit-like and arranged parallel to the axis of cell elongation, while in the others the cisternae were distended and appeared to contain a variable amount of electron dense material (Fig. 103). Both attached and free ribosomes were prominent. Whorls or curves of polysomes were found when the RER was tangentially sectioned (Fig. 104).

Figure 100. Two-week regenerating tendon. Electron micrograph showing portion of a fibroblast. The RER is well developed. The Golgi (G) apparatus is large, with a number of Golgi vesicles (v), some containing some electron dense material. Collagen (COL) fibres of the tendon are also shown. Durcupan.

X 42,600

Figure 101. Two-week regenerating tendon. Electron micrograph showing a Golgi vesicle. A halo (➡) can be seen between the vesicular membrane and the dense fibrillary content. Durcupan.

X 42,600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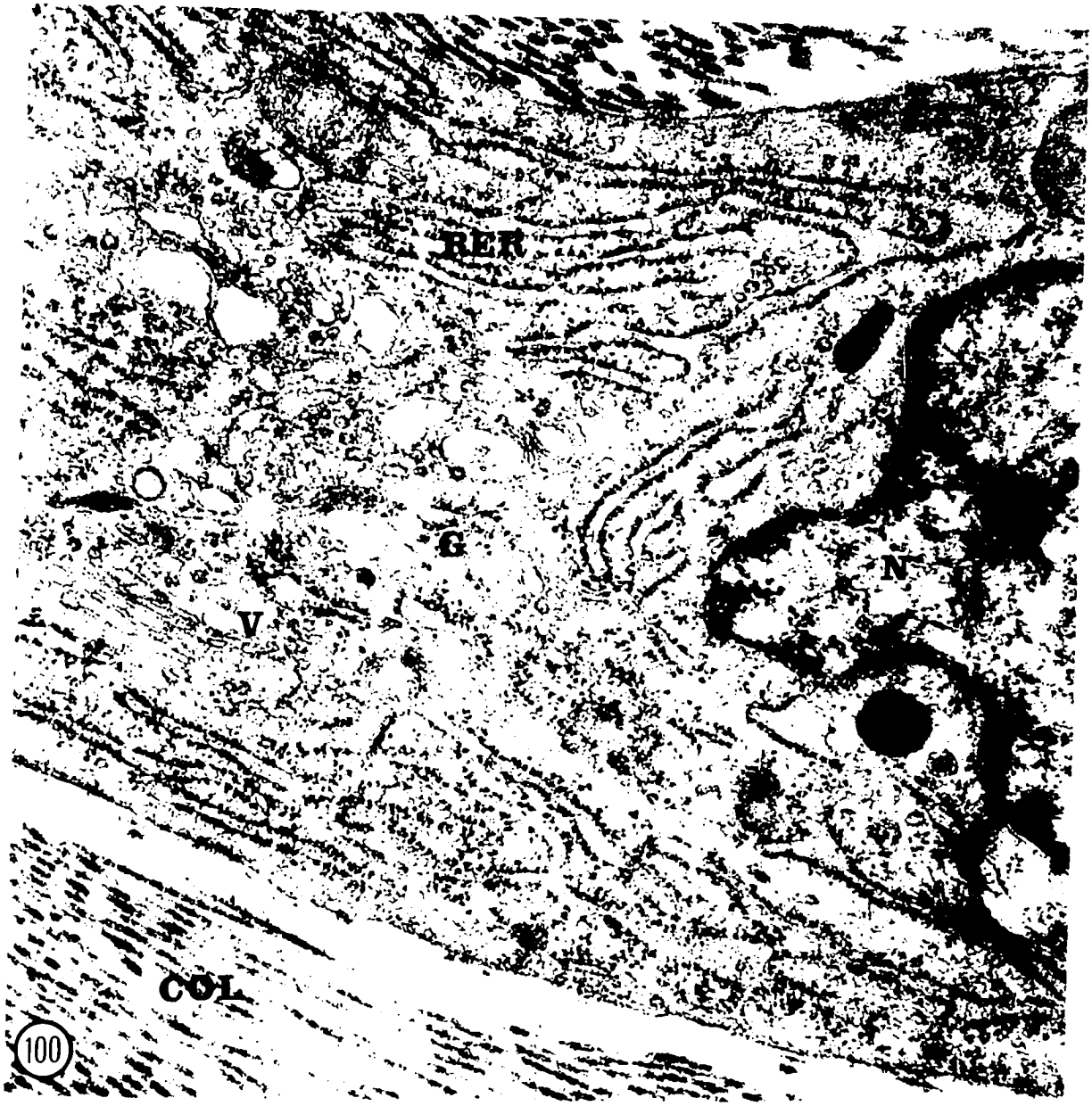

RER
G
N
V
COL
100

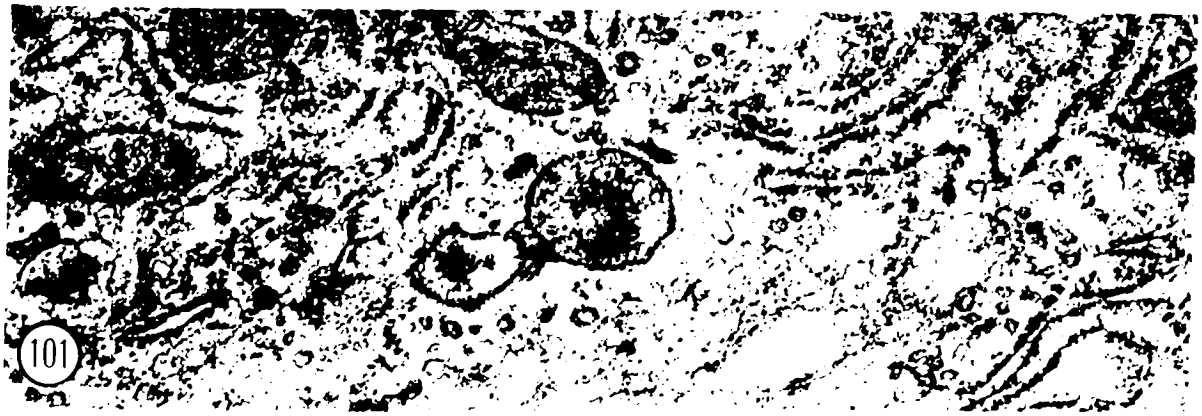

101

Figure 102. Fibroblast from two-week regenerating tendon. The arrows indicate collagen fibrils in the fibroblast. The lower group appear membrane-bounded while in the upper group the membrane is not discerned. The RER is very prominent and ribosome clusters are seen in the picture. At the region of the membrane (arrow) cut tangential to the surface distinct plasma membrane can not be seen. Cytofilaments (cf) are scattered in the cytoplasm. Durcupan.

X 25,400

Figure 103. Eight-day regenerating tendon. Portion of a fibroblast showing highly distended cisternae (DC) of the RER. Ribosomes are abundant. Durcupan.

X 20,570

102

103

102

111

Cytofilaments, about 50-100A° in diameter, a common feature of the fibrogenic cells (Fitton-Jackson, 1954; Kagikawa et al., 1959; Yardley, Heaton, Gaines and Shulman, 1960; Ross and Benditt, 1961; Ross, 1968; Göthlin and Ericsson, 1970), were also observed in the present investigation. These filaments were more prominent in some cells than in others. Some of them were randomly distributed, while others were concentrated at the cell periphery (Figs. 102, 104). In a section tangential to the cell surface, the cell membrane was not discernible and the filaments appeared to be in direct continuity with matrix (Fig. 102).

Other organelles such as vesicles, dense bodies, multivesicular bodies, microtubules, centrioles, spindle-shaped bodies (Voelz, 1964) and sometimes a cilium could be seen (Fig. 126).

In the early regenerating stage the cells were close together and had relatively few collagen fibrils between them. In late stages (from two weeks on) the cells were generally wide apart, and collagen fibrils of quite uniform size were prominent between fibroblasts. The collagen fibrils were about 30 mμ in diameter (Figs. 100, 102, 103, 104). Collagen fibers of larger calibre were found in older regenerating tendon.

(ii) Pre-cartilaginous stage

The cells were more oval in shape. Most of the long cytoplasmic processes were retracted. However, serrated or scalloped-

Figure 104. Fibroblast from eight-day regenerating tendon. A fibrillary zone (FZ) is seen just beneath the plasma membrane of the fibroblast. These filaments are also found in the cytoplasmic processes. The RER is very prominent and sheaths of ribosomes (R) can be seen in several regions. Durcupan.

X 26,550


104

104

shaped projections, characteristic of chondroblasts were observed. The cells might be single, or present in groups of two or three in a row, similar to the cell-nests observed in cartilage (Figs. 105, 106). The reason for these being classified in the pre-cartilaginous stage was that cartilaginous matrix could not be found in the immediate surroundings of the cells. Directly opposed to these cells were the collagen fibrils mentioned in the previous section.

The structure of the pre-chondroblast was quite similar to the fibroblast. Cytofilaments appeared to be more prominent than in the fibroblasts.

(iii) Cartilaginous stage.

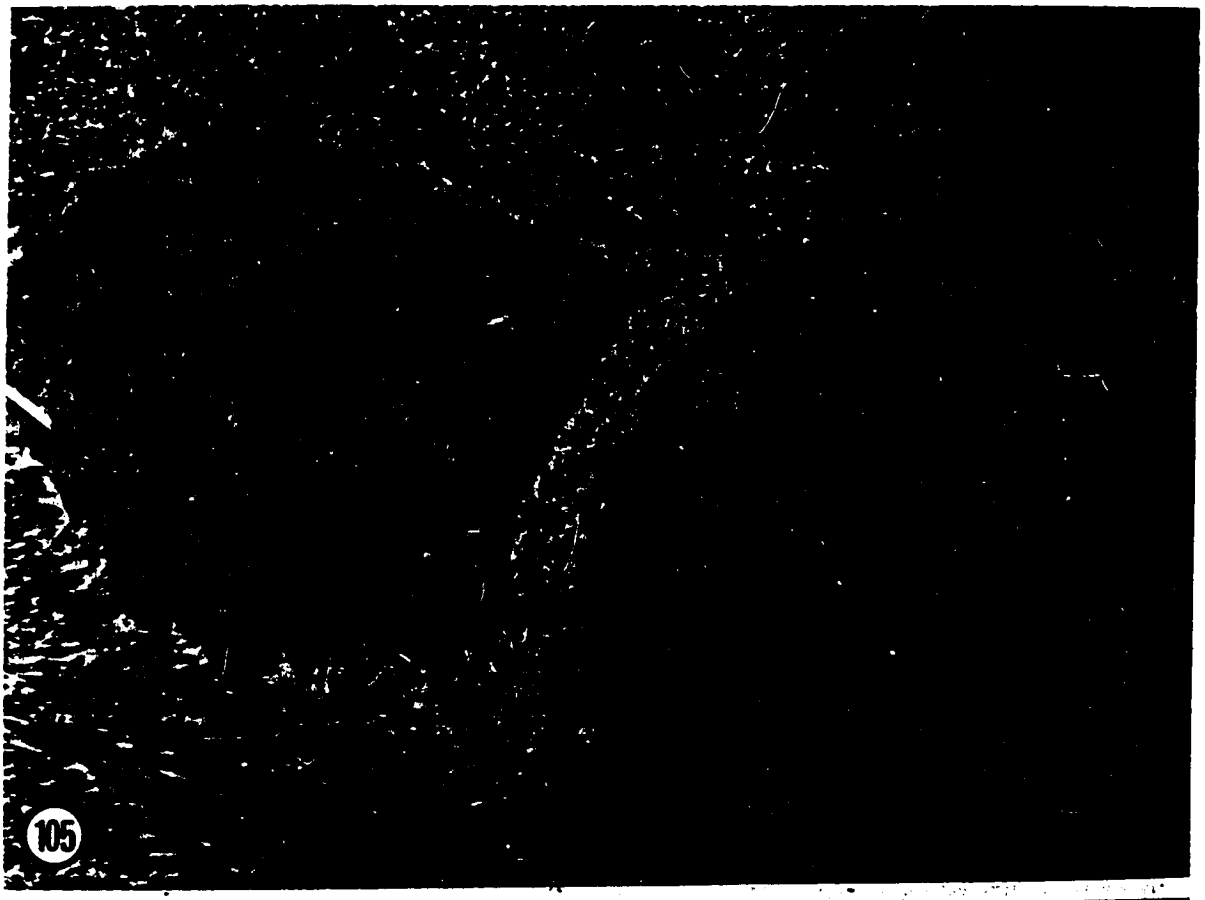
Typical cartilage cells are shown in Figures 107 and 108. The cells were oval in shape and the nuclei were eccentrically located. A very large GA was always present in juxtaposition to the nucleus. Large numbers of vesicles of varying size and shape were observed. Many of them had some electron dense material in them which formed a "halo" between this material and the vesicular membrane (Fig. 109). Some granules in the GA appeared to have a fuzzy coat. The nature of the smaller vesicles was not clear. However, from their relatively large size and fibrillary content they appeared to be the type derived from pinching off from the Golgi cisternae (Figs. 109, 110). The RER was very prominent. The cisternae might be slit-like and arranged in

Figure 105. Three-week regenerating tendon, showing two pre-chondroblasts. The cytoplasmic processes have retracted and the cells are ovoid in shape. Serrated edges, characteristic of chondrocytes, are seen in this case. The nucleus is ovoid in shape and has a thin rim of chromatin condensed beneath the nuclear membrane. However, typical cartilage matrix has not been produced. Durcupan.

X 10,560

Figure 106. Three-week regenerating tendon. A higher magnification picture showing part of two precondroblasts. The RER is very prominent. Few collagen fibres are seen between the pre-chondroblasts. Between cells some fine fibrillary material can be seen which is believed to be the early sign of matrix production. Durcupan.

X 40,700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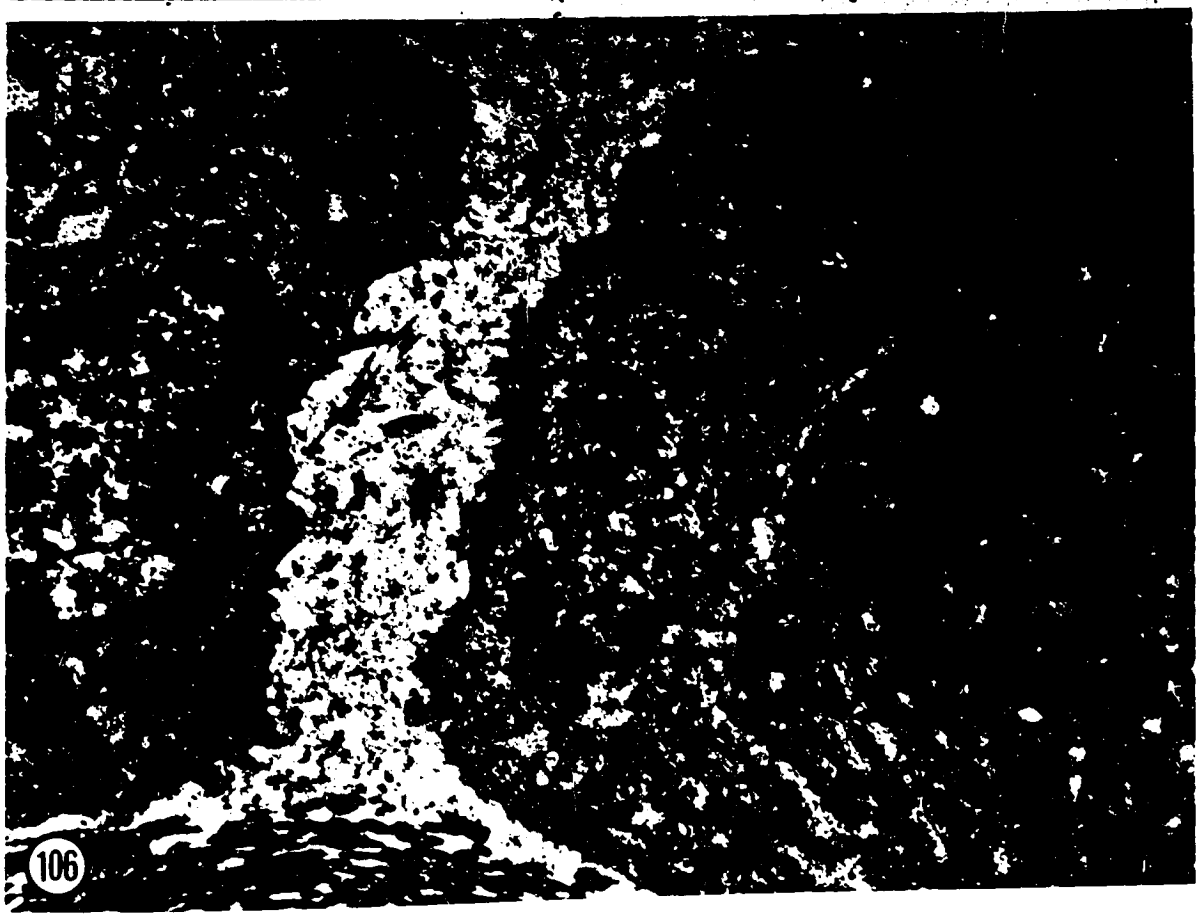
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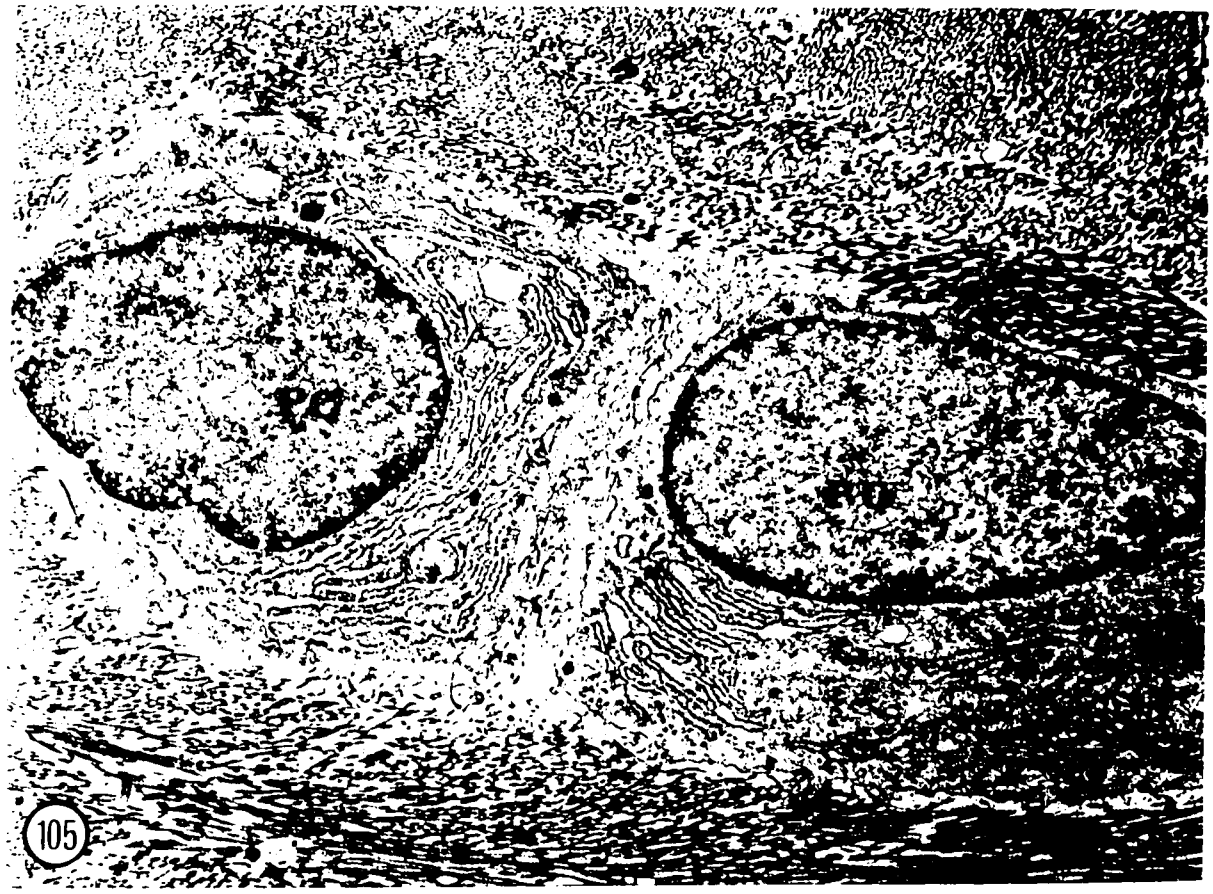
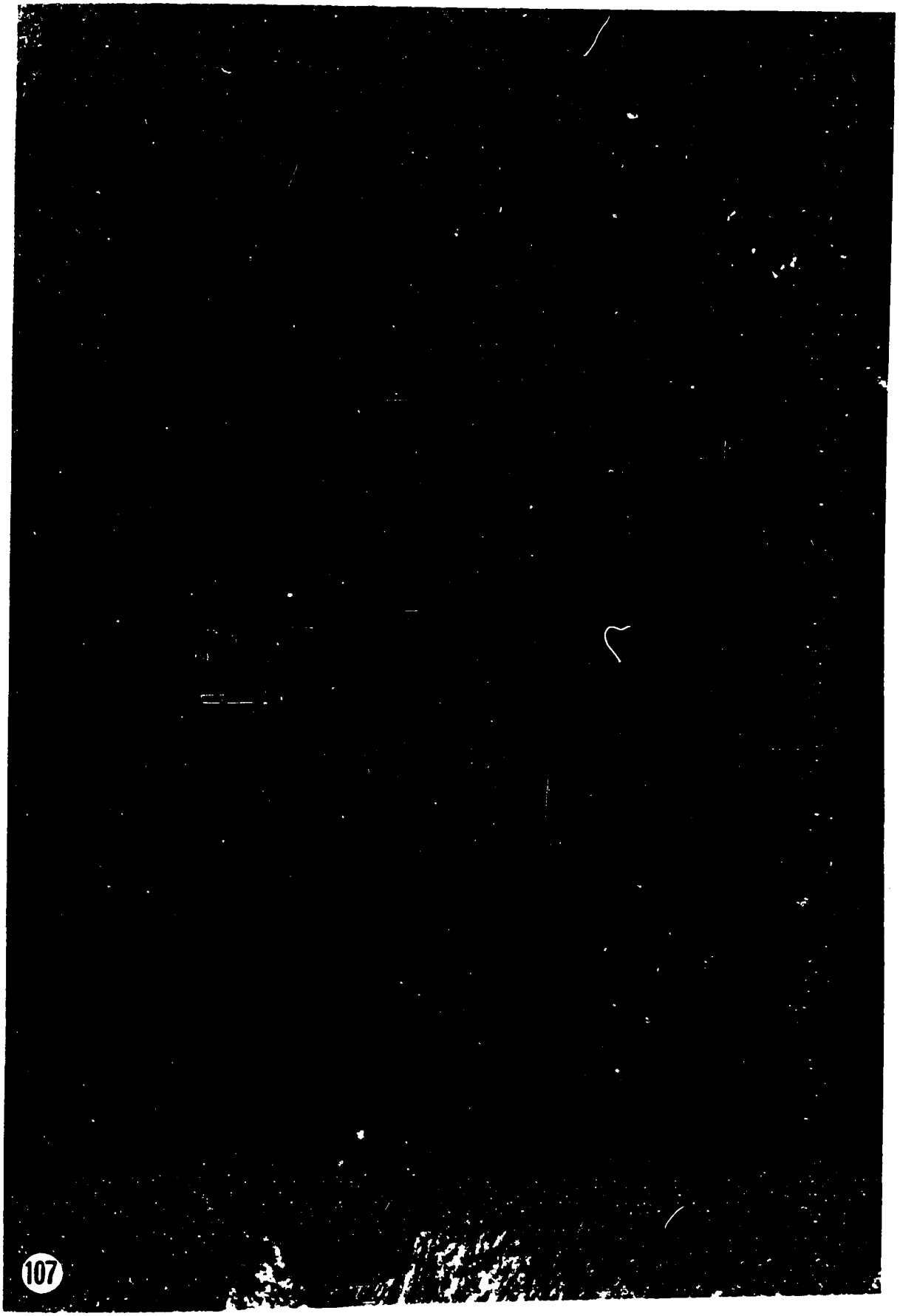


105

106

Figure 107. Survey picture of an area of metaplastic cartilage in 41-day regenerating tendon. Typical cartilage cells are seen in this region. Collagen fibres (COL) are found interspersed with the cartilage matrix (MAT) between chondrocyte. The cartilage matrix is quite abundant in this particular picture. Vestopal.

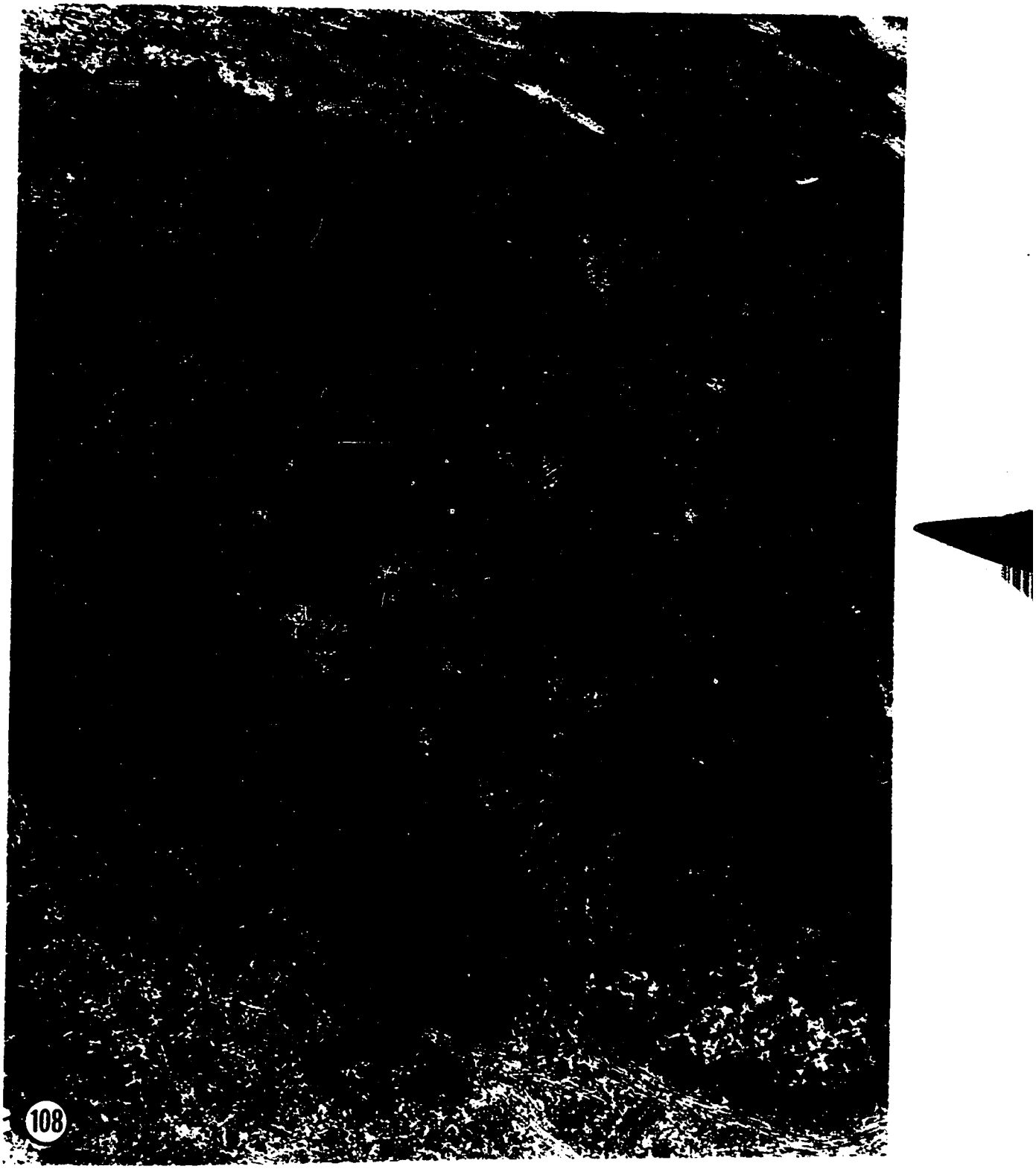
X 10,150


107


MAT
COL

Figure 108. A chondrocyte from a four-week regenerating tendon. Large number of cytofilaments (Cf) are found in the cytoplasm, especially in the perinuclear position. The RER is prominent. Collagen fibres are abundant outside the chondrocyte and only a small rim of typical cartilage matrix is seen between chondrocyte and collagen of the tendon. Epon.

X 25,830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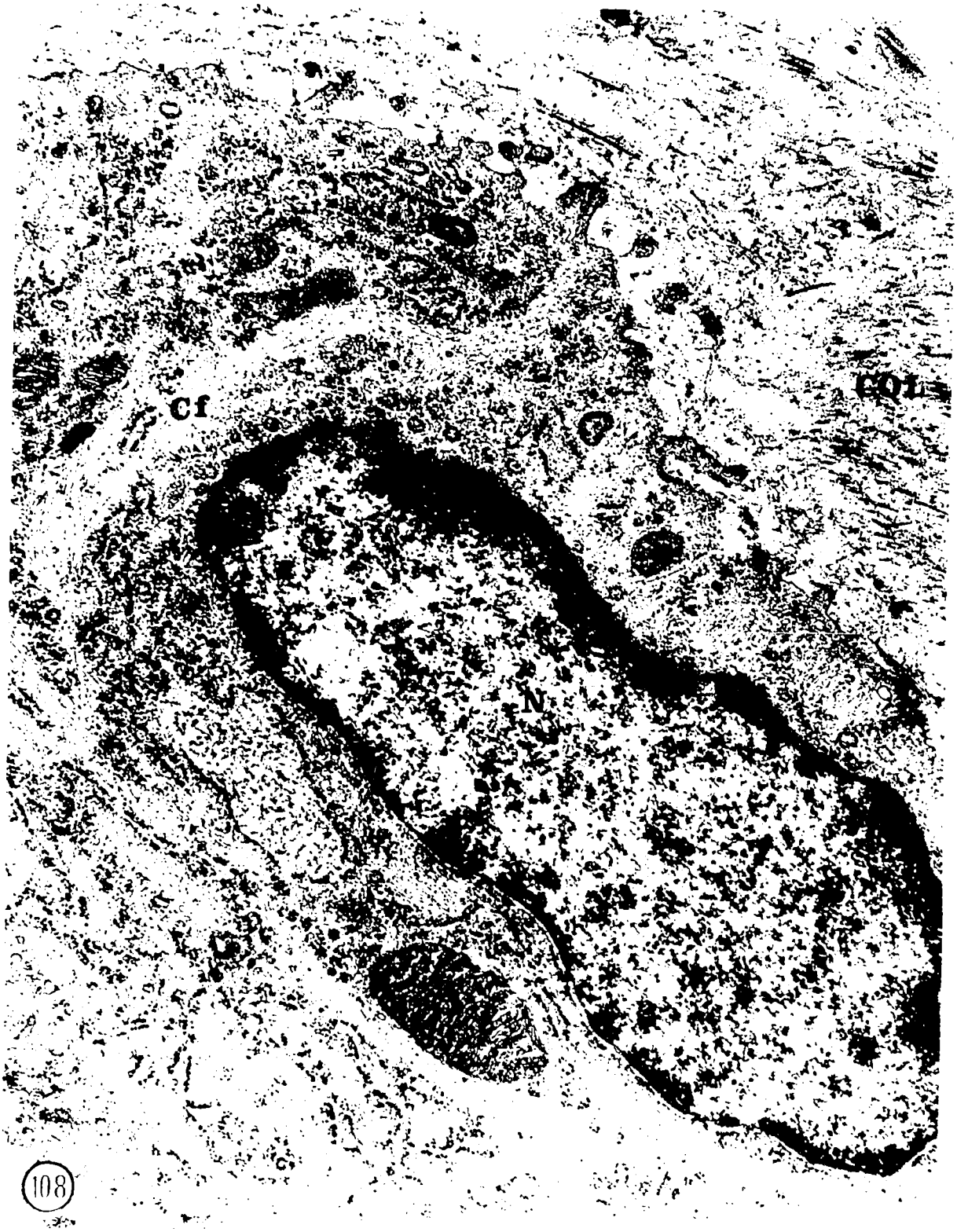
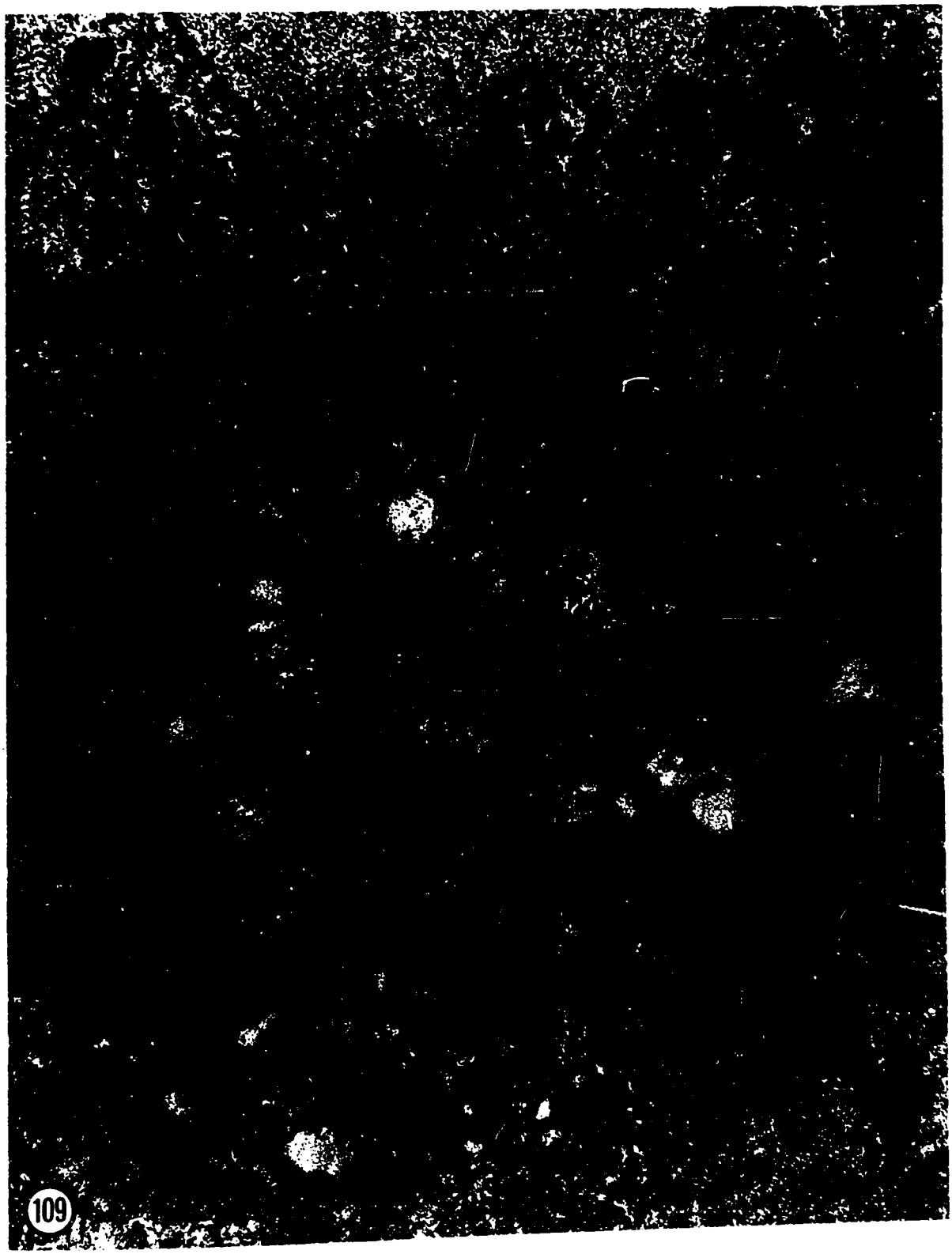


(108)

Figure 109. Three-week regenerating tendon, showing a highly elaborate. Golgi apparatus in a chondrocyte of the metaplastic cartilage. Stacks of Golgi membranes and large numbers of various sized Golgi vesicles (v) are seen. Many of the larger ones (v) contain dense fibrillary material separated by a "halo" from the vesicular membrane. The RER surrounding the Golgi is highly distended (DC) with finely granular material. Epon.

X 64,000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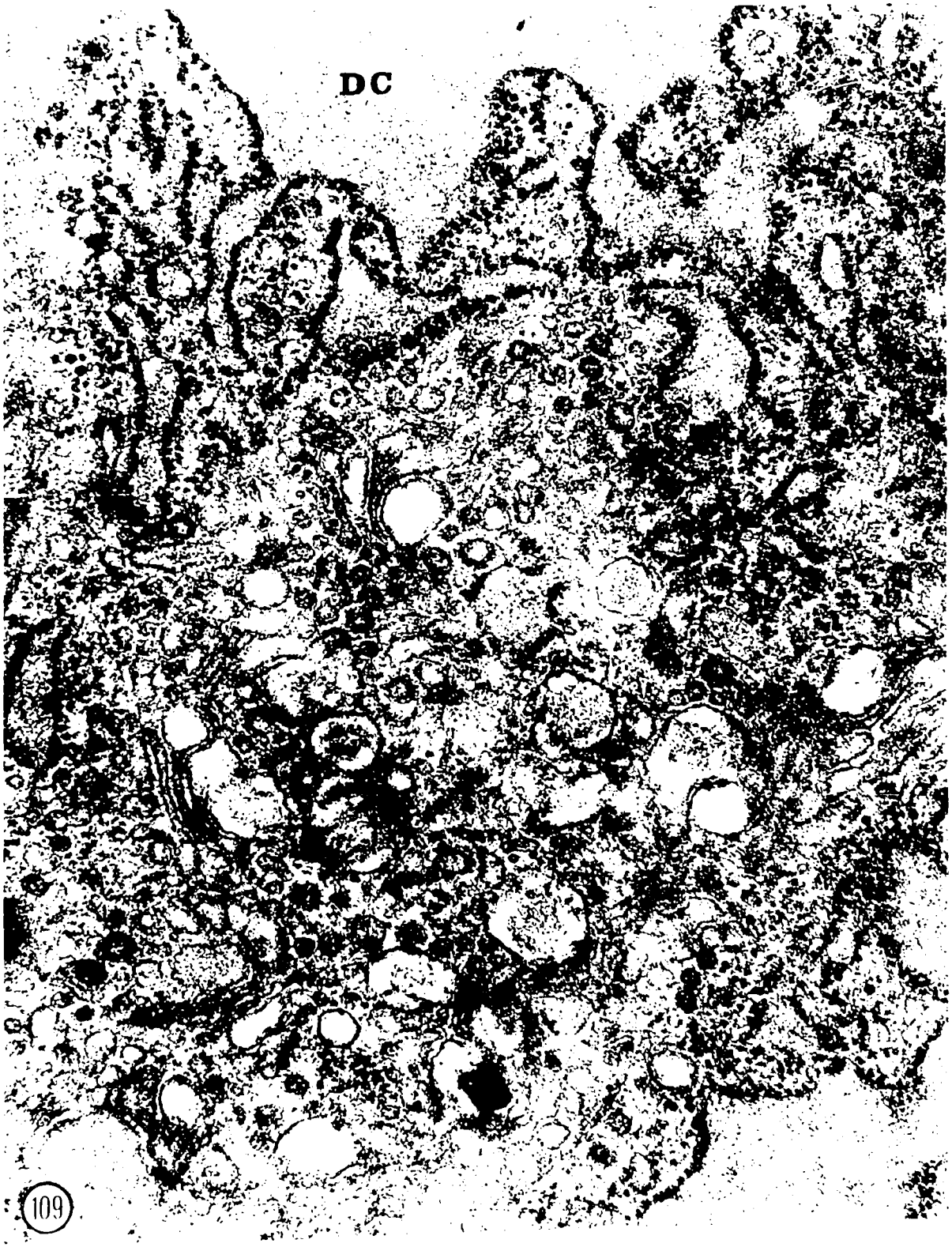


109

Figure 110. Golgi apparatus of another metaplastic cartilage cell from three-week regenerating tendon. A vesicle (►◄) is pinching off from the Golgi stack membranes. Vesicles of various sizes including the dense core vesicles (v) are also shown. Epon.

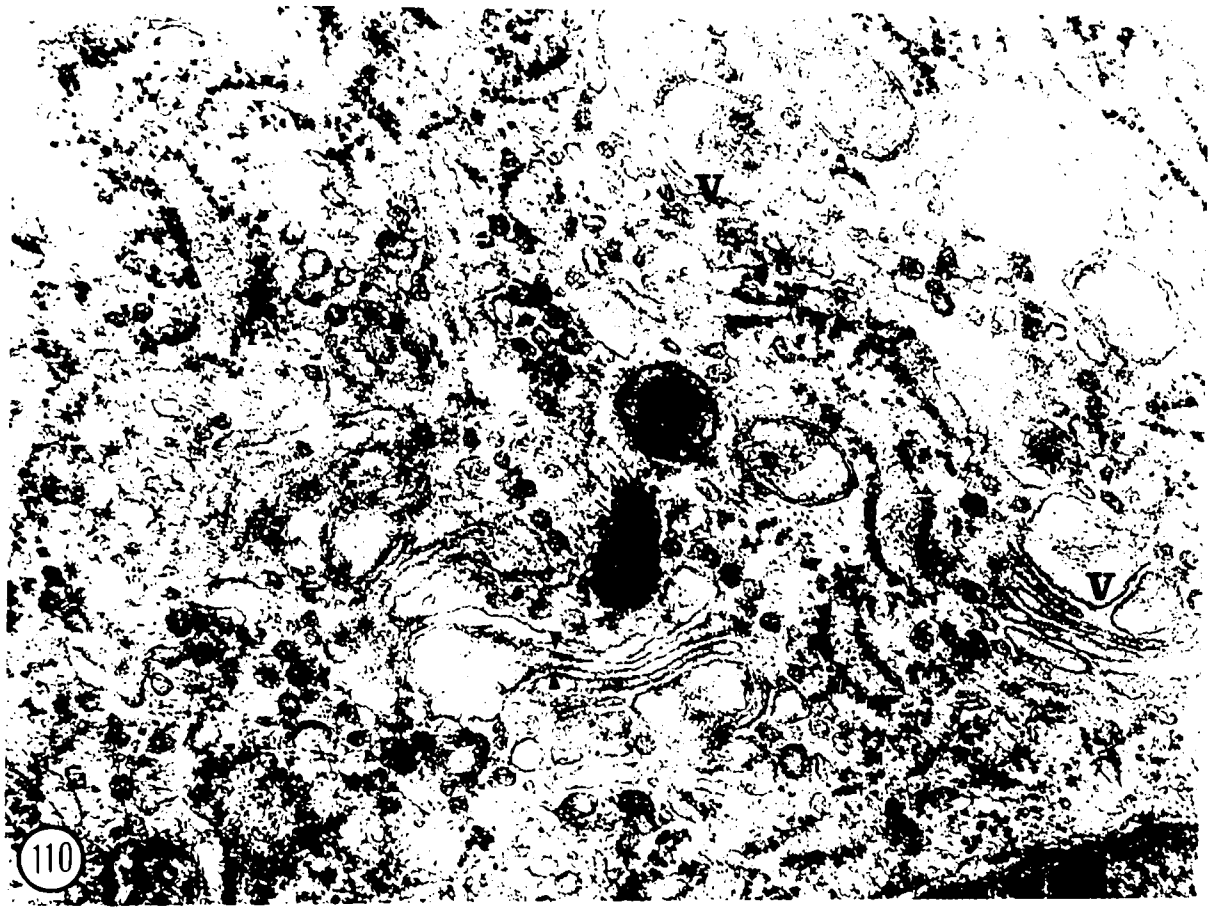
X 60,800

Figure 111. Three-week regenerating tendon, showing stacks of RER in a metaplastic cartilage cell. Finely granular material is seen in the cisternae of the RER. Cytofilaments can be seen between membranes of RER. Epon.

X 44,800

110

111



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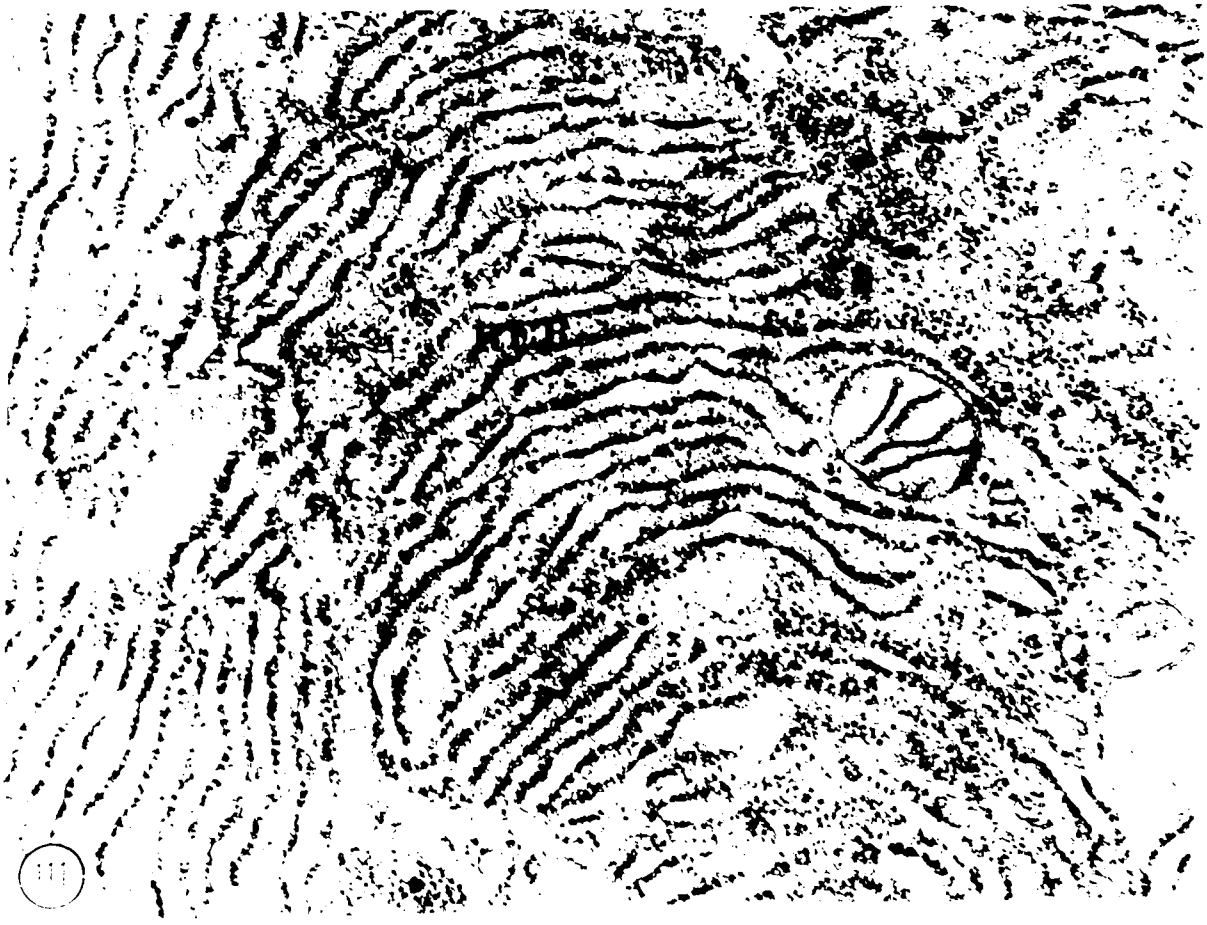


111

stacks in some places (Fig. 111), while highly distended in others.

A very striking feature of the chondrocytes observed here was the presence of an enormous number of cytofilaments (Figs. 108, 112). Some of them were randomly distrubuted; others were regularly arranged in bundles. They might be found between RER, but quite frequently they formed a fibrillary band, which partially enclosed the nucleus (Fig. 108). These filaments were of about 100A° in diameter and appeared larger than the filaments of the fibrillary band at the periphery of the cartilage cells (Figs. 113, 114). There were usually no ribosomes in the regions occupied by bundles of cytofilaments (Figs. 108, 112).

The peripheral fibrillary zone of the chondrocyte was wide and prominent. The filaments were irregularly arranged and, in many cases, the cell membrane was obscured by this (Fig. 113). Vesicles of various sizes, both with and without fibrillary contents, could be seen (Figs. 113, 114). Some of them appeared to be discharging their contents extracellularly.

Glycogen granules present in groups were observed in some cells.

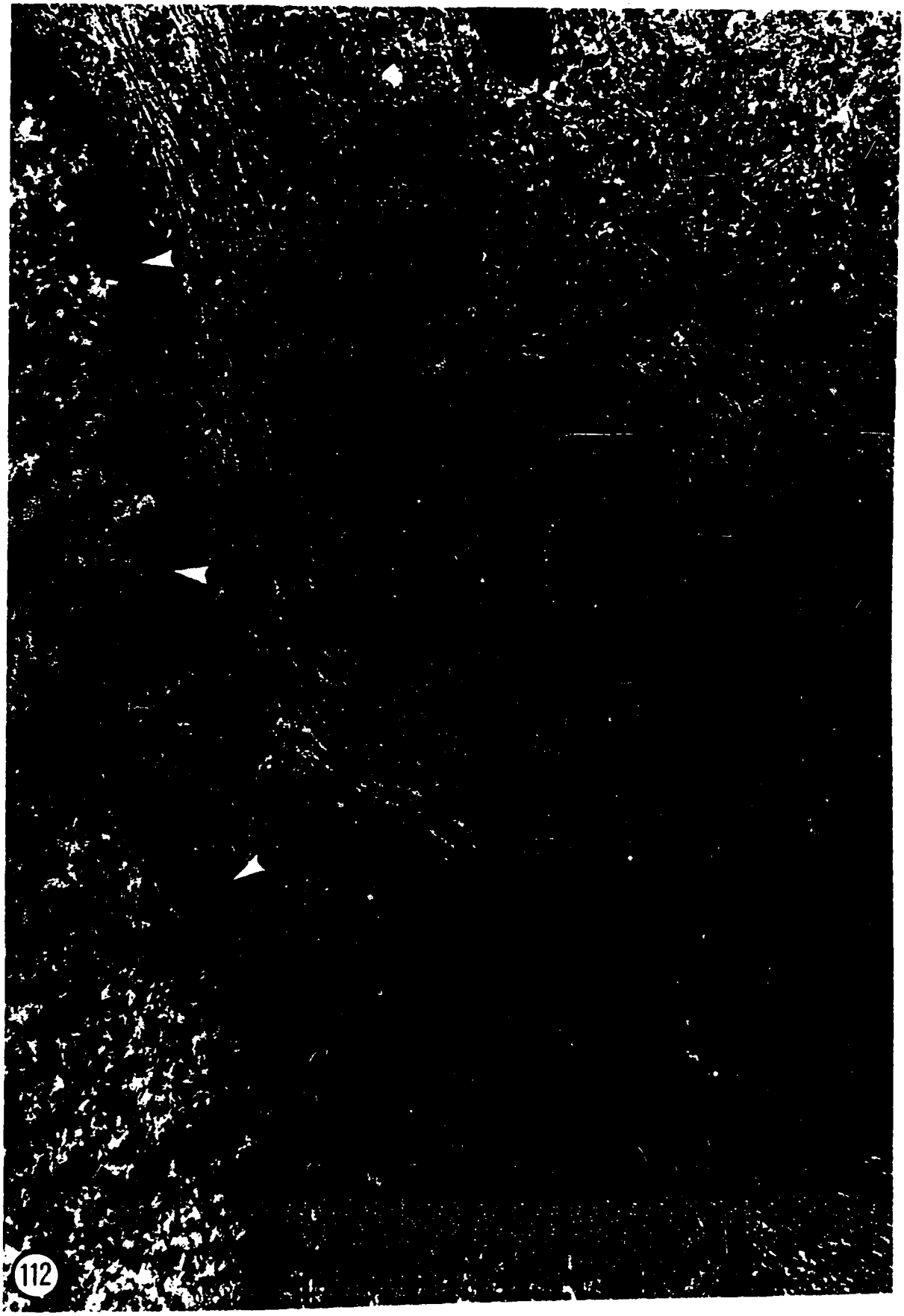
Mitochondria were oval in shape, some having mitochondrial granules.

Other organelles such as dense bodies, microtubules, and multivesicular bodies were also observed.

The chromatin of the nucleus was condensed to form a band beneath the inner membrane, except at the region of nuclear pores (Fig. 112).

Figure 112. Three-week regenerating tendon. Enormous number of cytofilaments (Cf) are found in this chondrocyte. Each cytofilament is about 80-100A° in diameter. Many of them aggregate to form bundles of cytofilaments. Others are scattered singly in the cytoplasm. The chromatin (►) of the nucleus (N) is condensed to form a homogenous band against the inner nuclear membrane. Epon.

X 65,600


112

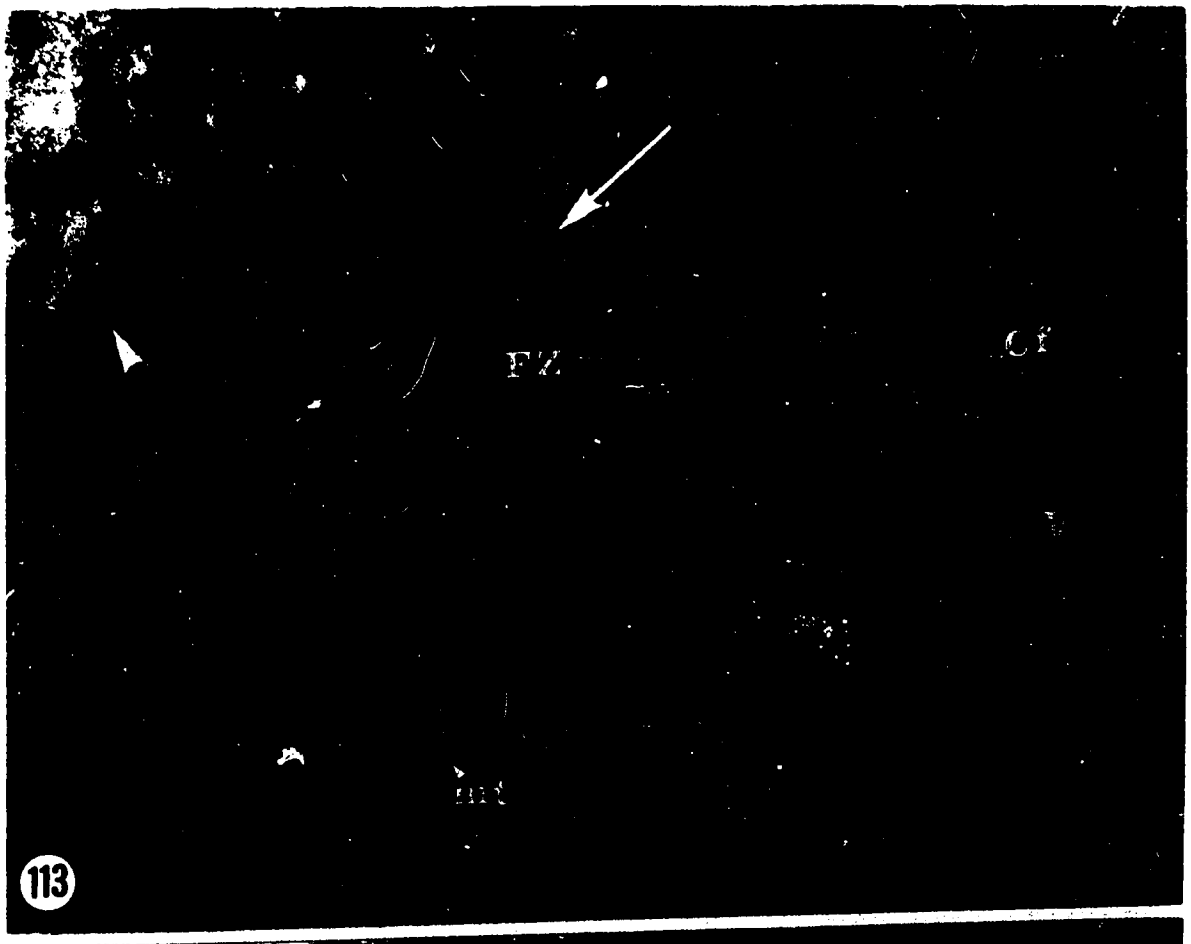
112

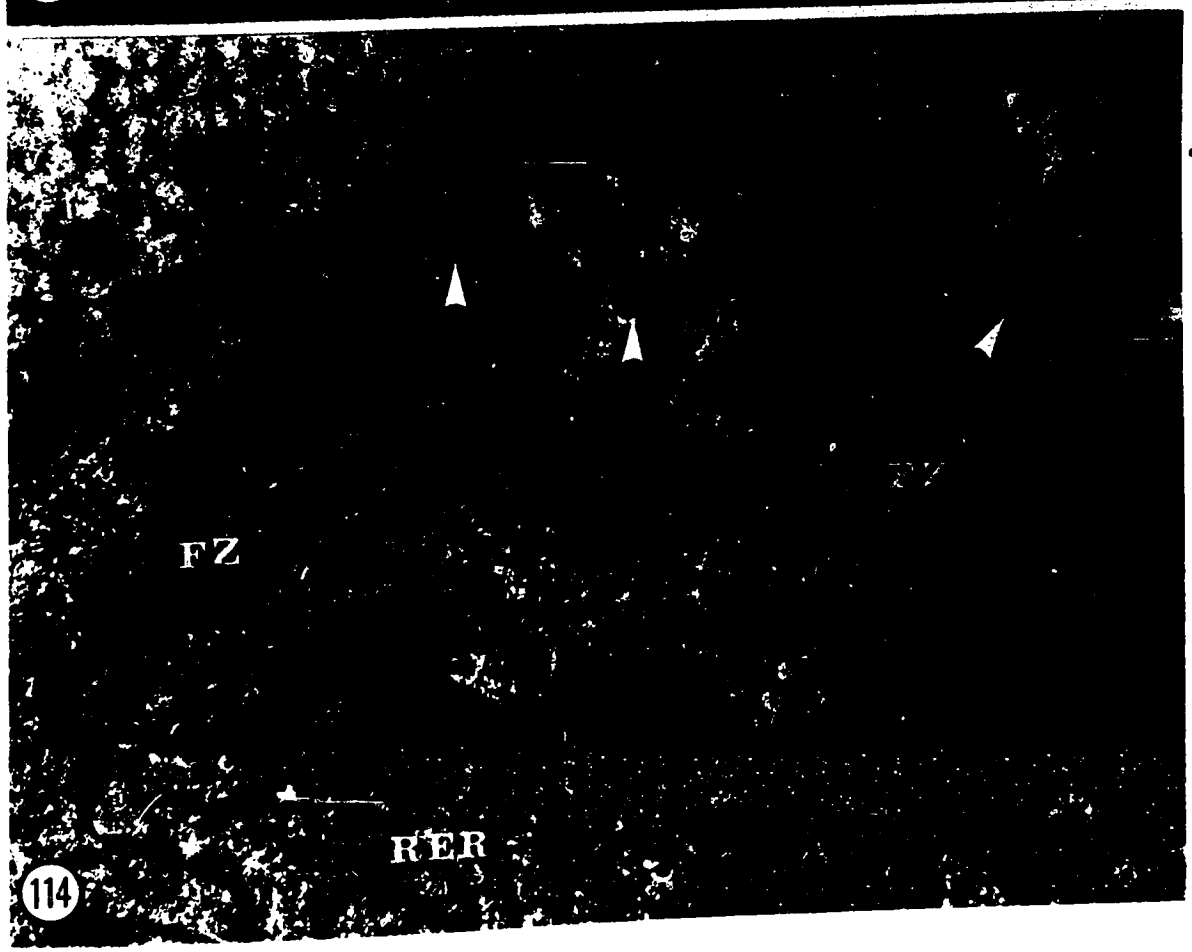
Figure 113. Three-week regenerating tendon. The fibrillary zone (FZ) is made up of fine randomly distributed filaments of sizes ranging from 50A° to 80A°. The cytofilaments (cf) which are more regularly arranged are slightly larger than the filaments at the fibrillary zone. Small coated vesicles (➤) are found in the fibrillary zone. mt, microtubule. At the point indicated by an arrow, the fibrillary zone appears continuous with the extracellular matrix filaments. Epon.

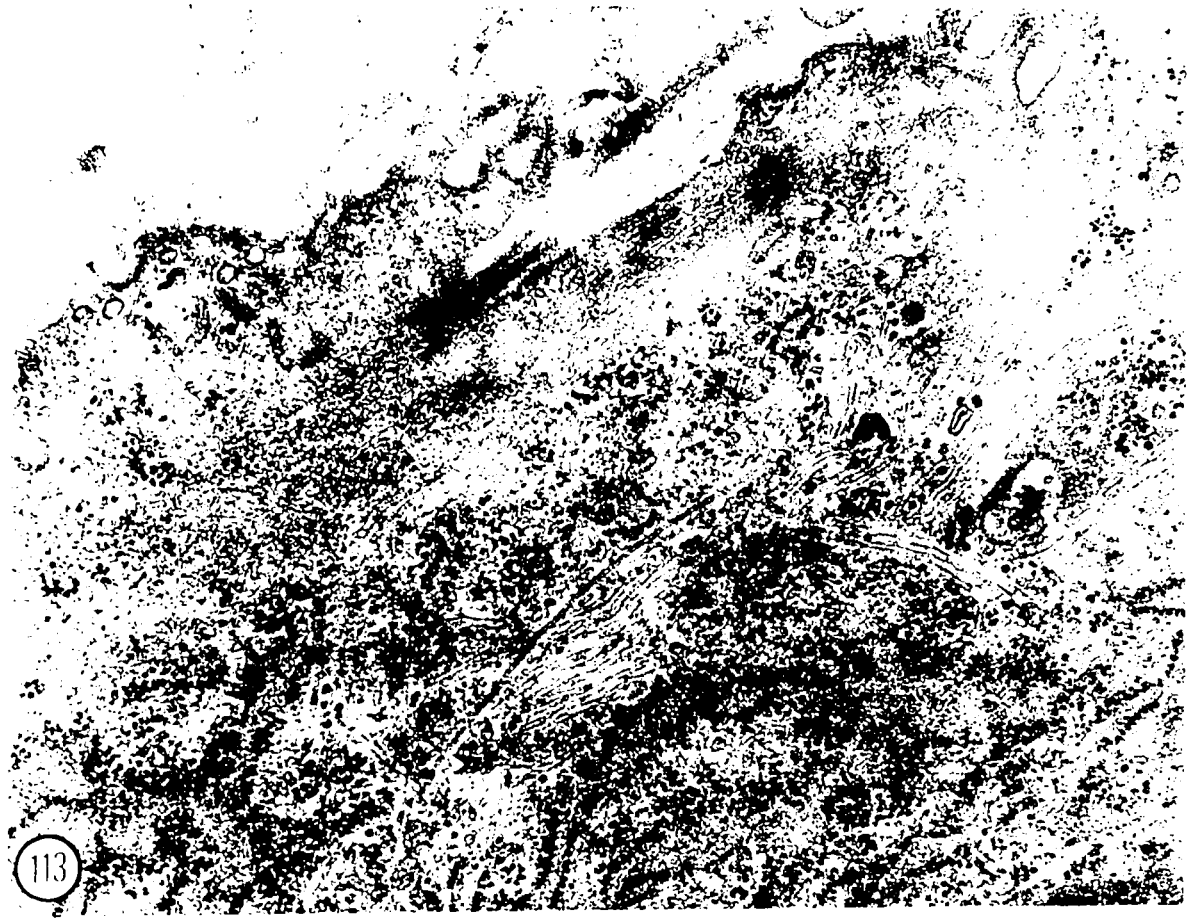
X 46,400

Figure 114. Three-week regenerating tendon, showing thick fibrillary zone (FZ) of a chondrocyte. Many coated vesicles (➤) are seen beneath the plasma membrane. A thin rim of typical cartilage matrix is seen outside the chondrocyte. Epon.

X 52,800


FZ
113


FZ
FZ
RER
114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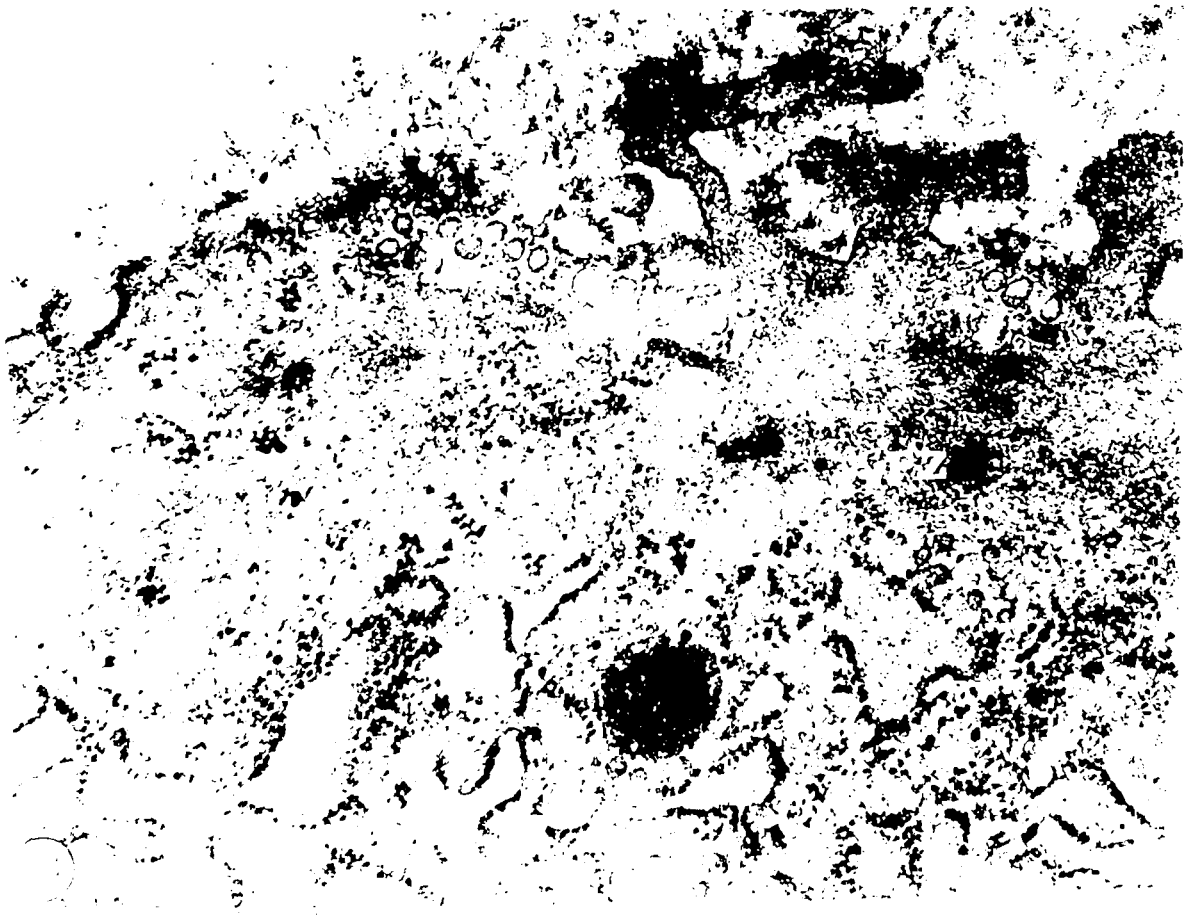


The matrix of the metaplastic cartilage was indicated by a relatively clear space immediately outside the chondrocyte. The matrix might be abundant in some areas of metaplastic cartilage, while in others it might be just barely discernible. At high magnification fine, randomly distributed fibrils could be seen in the matrix. Although some collagenous fibrils could still be seen in the immediate surroundings of the chondrocytes, most of them were of the same calibre as those in the cytoplasmic vesicles referred to earlier (Figs. 109, 111, 116). In several cases, these fibrils were condensed just outside the cell membrane to form a larger, dense, image, which bore a resemblance to the basement membrane of epithelial cells (Fig. 115).

In other regions bundles of non-periodic fibrils were observed in the lacunae.

Moving away from the clear matrix, fine fibrils became intermingled with the collagen fibrils. The collagenous fibrils found there were mostly smaller than those of non-metaplastic sites, and they appeared to be randomly and more loosely arranged.

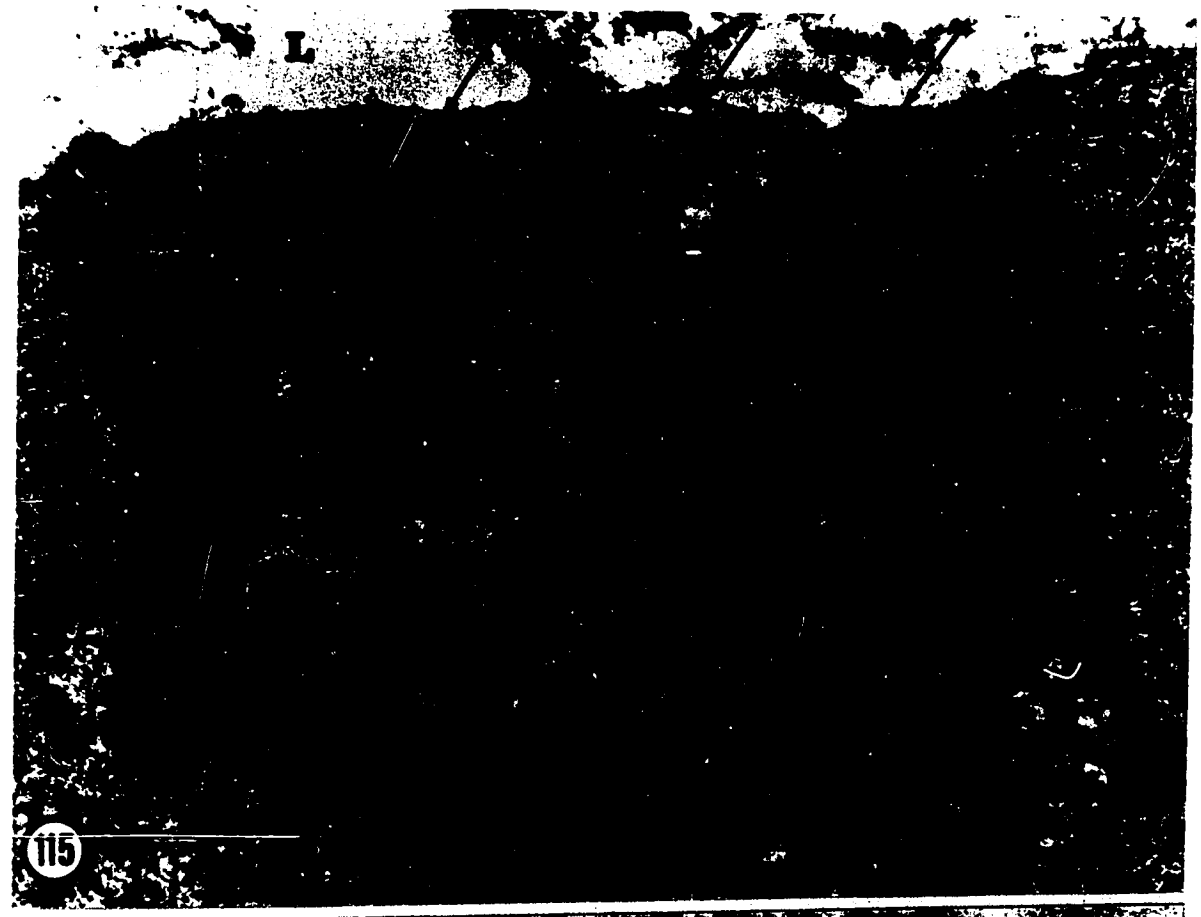
A peculiar type of vesicle was also observed in the matrix of the metaplastic cartilage, similar to that in normal epiphyseal cartilage (Anderson, 1969; Thyberg and Friberg, 1970). These were round, ovoid, or irregular in shape and varied from 350A^{o} to 1-2 μ. Some of them were made up of packages of unit membrane; others were membrane-bounded, or partially membrane-bounded, with various amounts of dense material in them. Some of them contained ribosomes, or cytofilaments, or mitochondria, or all of these (Figs. 117, 118). Smaller, non-membrane-bounded, dense granules as described for matrix of cartilage (Godman and Porter, 1960; Revel and Hay, 1963; Takuma, 1960;

Figure 115. Three-week regenerating tendon. The fibrillary material of the cartilage matrix sometimes condenses just outside the cell membrane to form a layer of high density (arrows) bearing a resemblance to the basement membrane of epithelial cells. Epon.

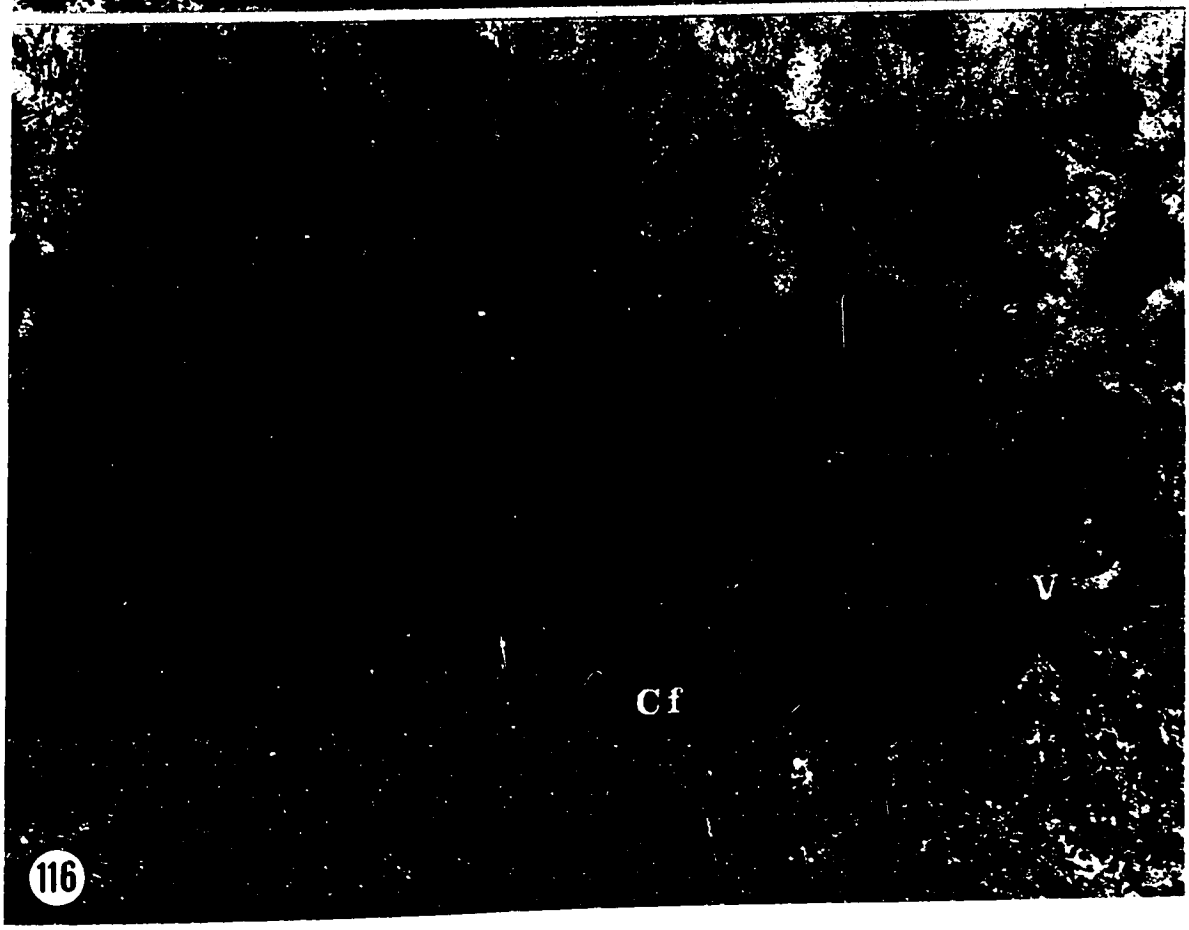
X 29,000

Figure 116. Three-week regenerating tendon, showing a cartilage matrix and a portion of a chondrocyte. The matrix in this particular specimen is composed of matrix filaments or fibrils, the sizes of which are comparable to the filaments in the dense core vesicles (v) of the chondrocyte. Epon.

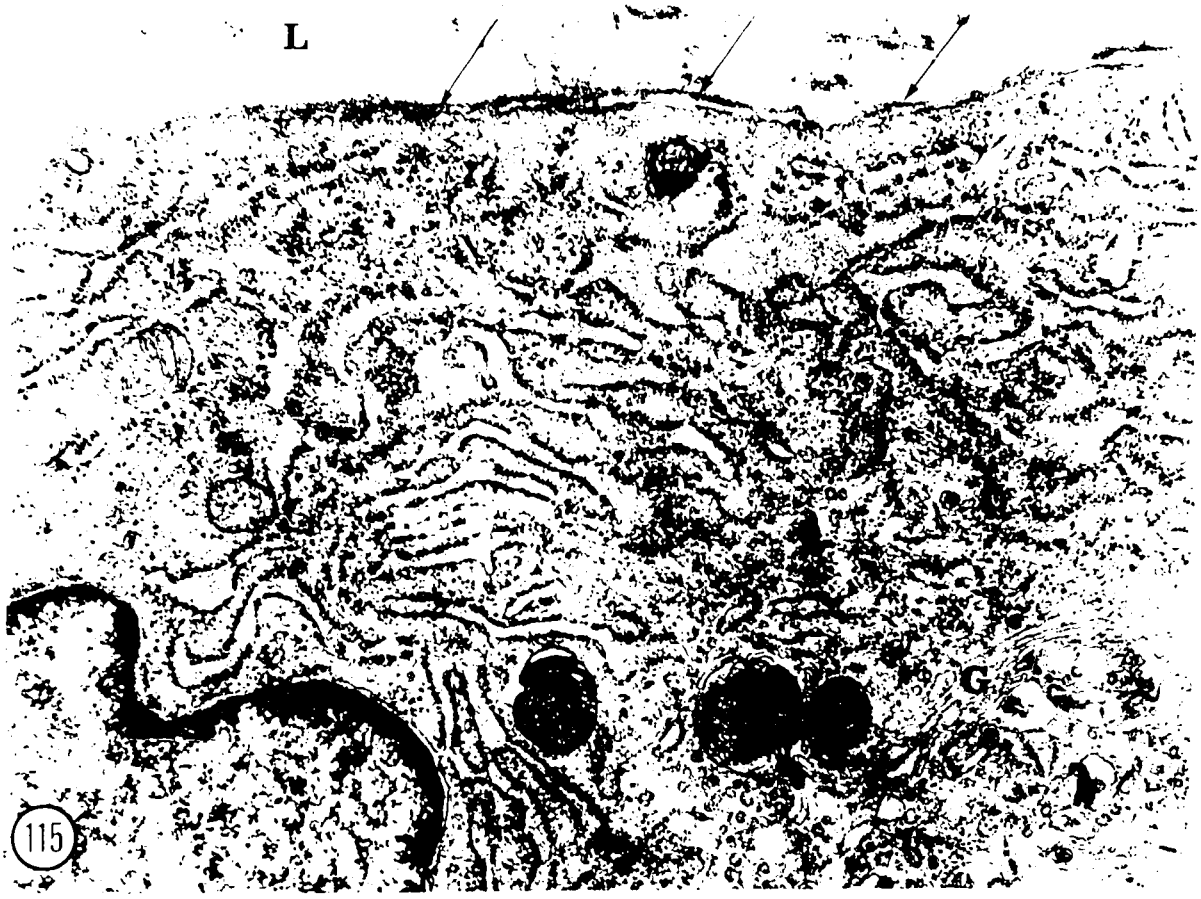
X 49,600



115



116


L
G
115

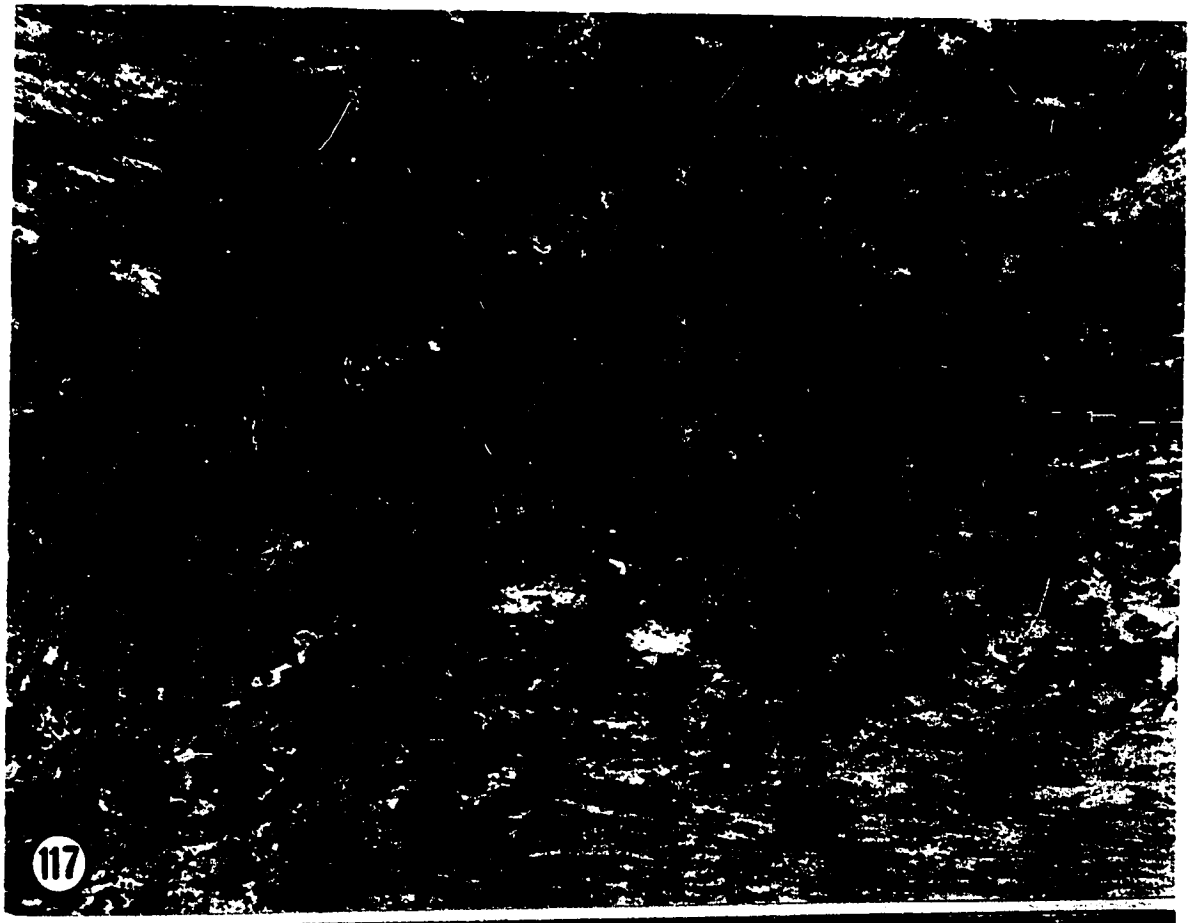

MAT
1h

Figure 117. Four-week regenerating tendon. A large number of matrix vesicles (Mtv) is seen embedded in the mainly collagenous cartilage matrix. Matrix vesicles of this picture are mainly small and do not contain any cytoplasmic organelles. Matrix fibrils are more abundant on the left than on the right sides of the picture. Epon.

X 30,000

Figure 118. Three-week regenerating tendon. A large and many small matrix vesicles (Mtv) are shown. Within the large matrix vesicle, mitchondria, cytofilaments and coated vesicles can be visualized. Epon.

X 49,600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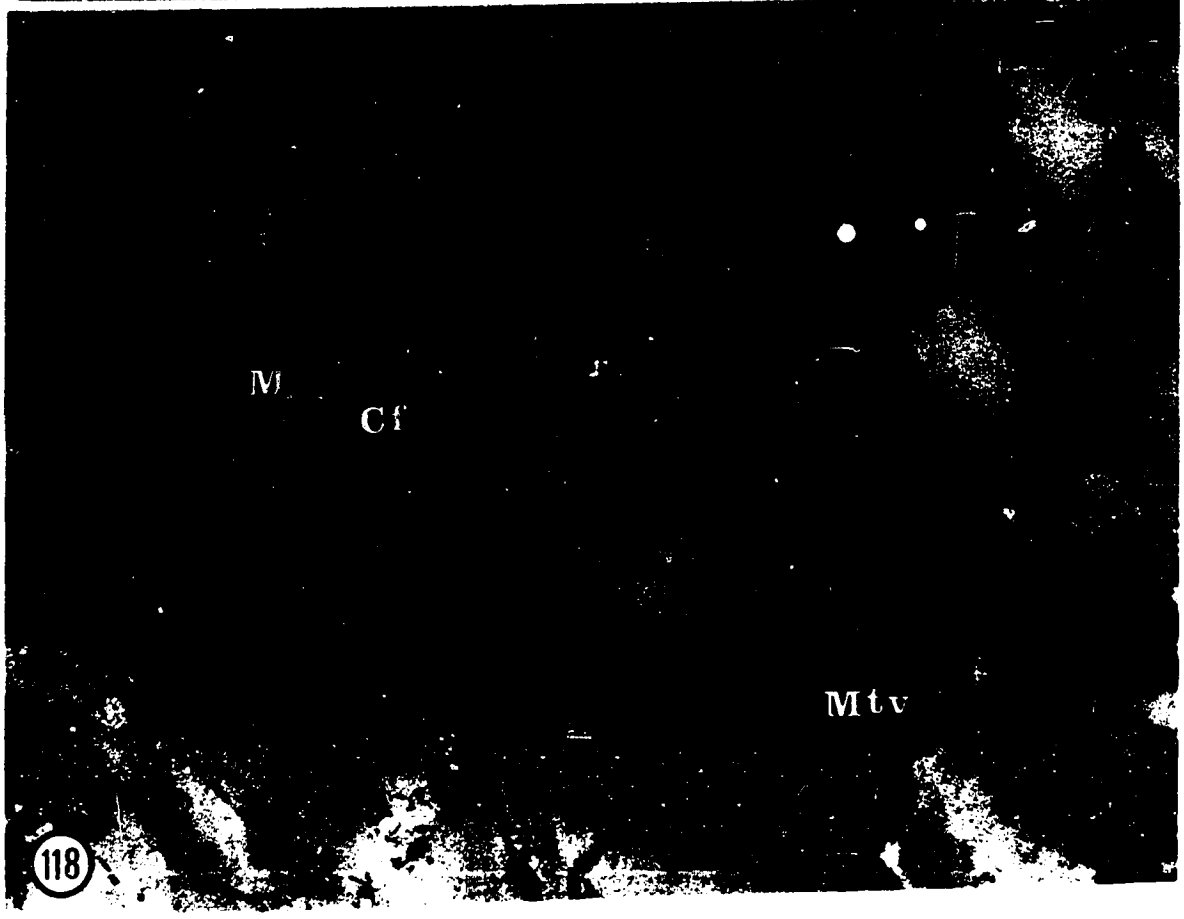

M
Cf
Mtv
118

117

118

Matukas, Panner and Orbison, 1966; Khan and Overton, 1970) were scattered throughout the matrix and also the lacunae (Figs. 117, 119).

Most of the matrix vesicles were distributed some distance from the chondrocyte and very often in groups. In some cases, these seemed to be closely related to the chondrocyte (Fig. 120).

(iv) Calcified cartilage

Early stages of calcification were observed as radiating clusters of electron dense, needle-like crystal deposited in the matrix (Fig. 121). Quite often one or two collagen fibrils might be studded by these crystals, while all the others remained unaffected. In other cases, the apatite crystals might not have any association with collagen fibrils. No spacial relationship of apatite crystals and matrix vesicles was observed. In late stages, these calcification sites spread and the crystals formed a confluent mass (Fig. 122).

In the early stages of calcification, the cartilage cells were normal. Later, however, hypertrophy and degeneration of the cells were observed (Fig. 123).

8. Cilia

During the study of metaplasia, cilia have been observed in cells which are normally without them. The number of cilia found in the various tissues is summarized in Table 3.

In squamous metaplasias, that is, in trachea and urinary bladder, single cilia were found in spinous and basal cells (Fig. 124). They were usually short and projected into the cytoplasm or intercellular spaces.

Figure 119. Four-week regenerating tendon. A high magnification micrograph showing the matrix of the metaplastic cartilage. Matrix fibrils, matrix granules (MG), and matrix vesicles (Mtv) are shown. Epon.

X 67,200

Figure 120. 41-day regenerating tendon, showing the close relationship of matrix vesicles (Mtv) and the chondrocyte (c). A band of vesicles is extending out from the cell to the matrix. Vestopal.

X 22,680


119


120

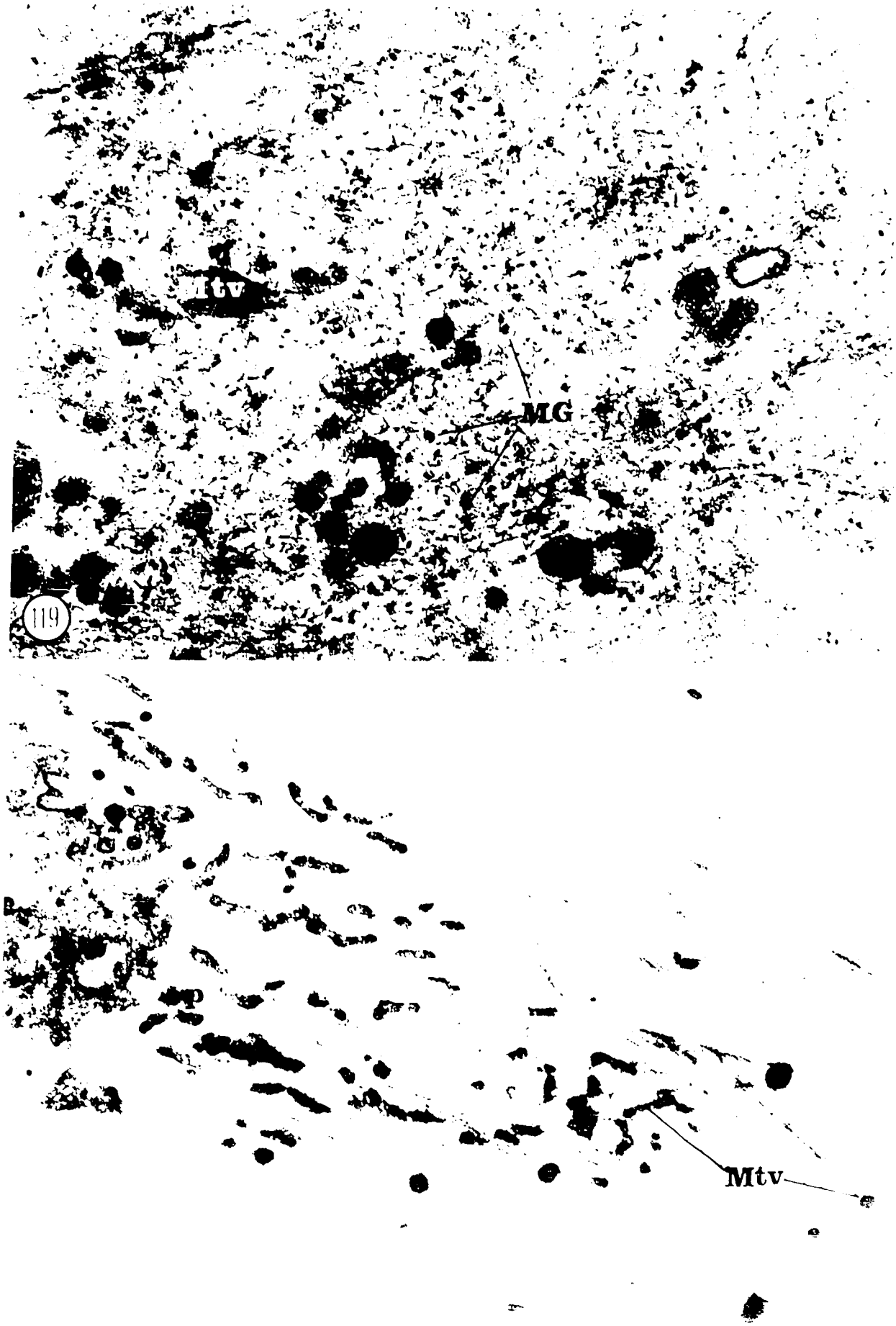

Itv
MG
119
Mtv

Figure 121. 41-day regenerating tendon. Early calcification of the metaplastic cartilage. Needle-shaped crystallites (CRY) in clusters are randomly distributed through the predominately callagenous matrix. Vestopal.

X 10,750


121

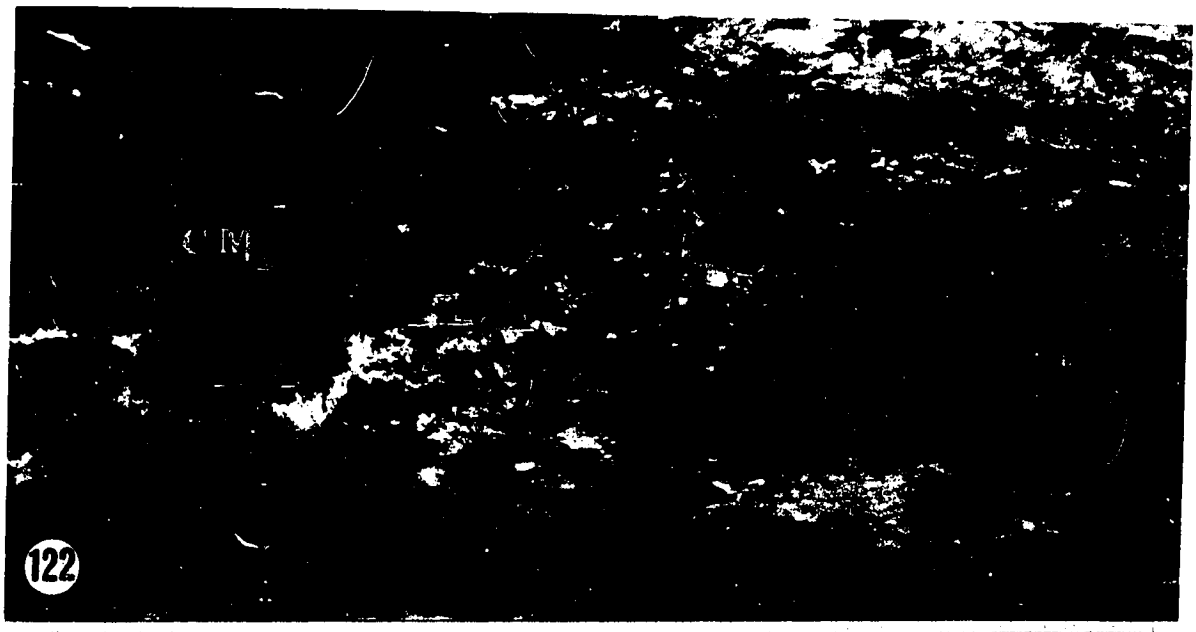

CRY
C

Figure 122. 41-day regenerating tendon. Calcified matrix of metaplastic cartilage. A confluent mass of calcified matrix (CM) is shown on the left, while small clusters of crystallites are on the right. Vestopal.

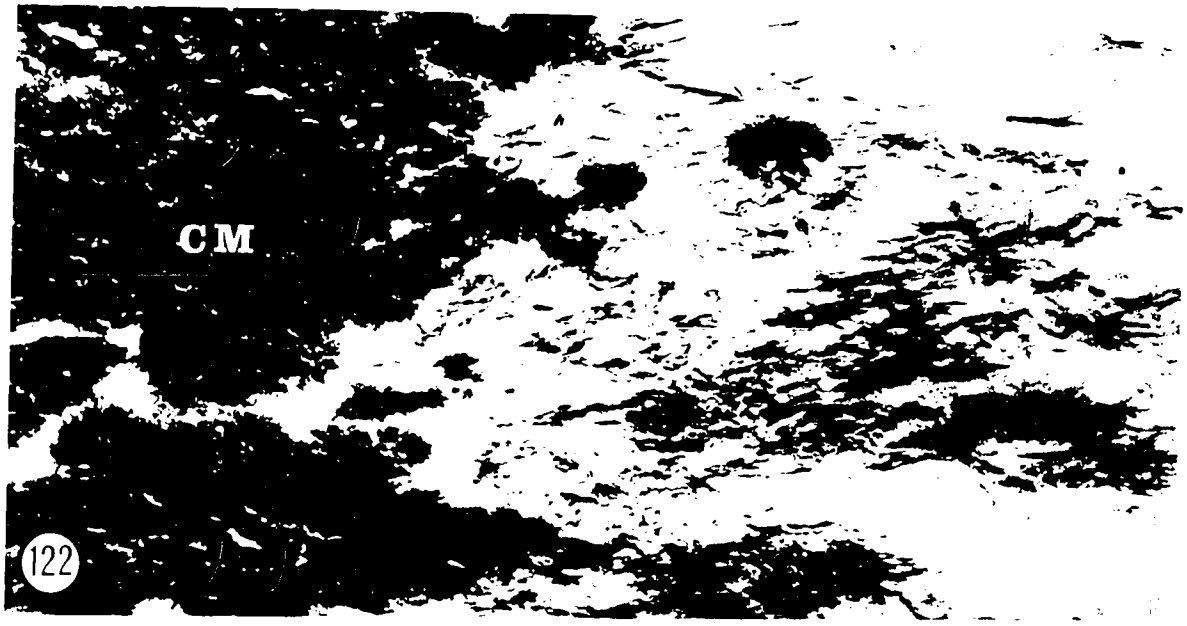
X 14,000

Figure 123. 41-day regenerating tendon. A degenerating chondrocyte (DEG) within the calcifying matrix (CM). Vestopal.

X 8750


CM
122


DEG
123



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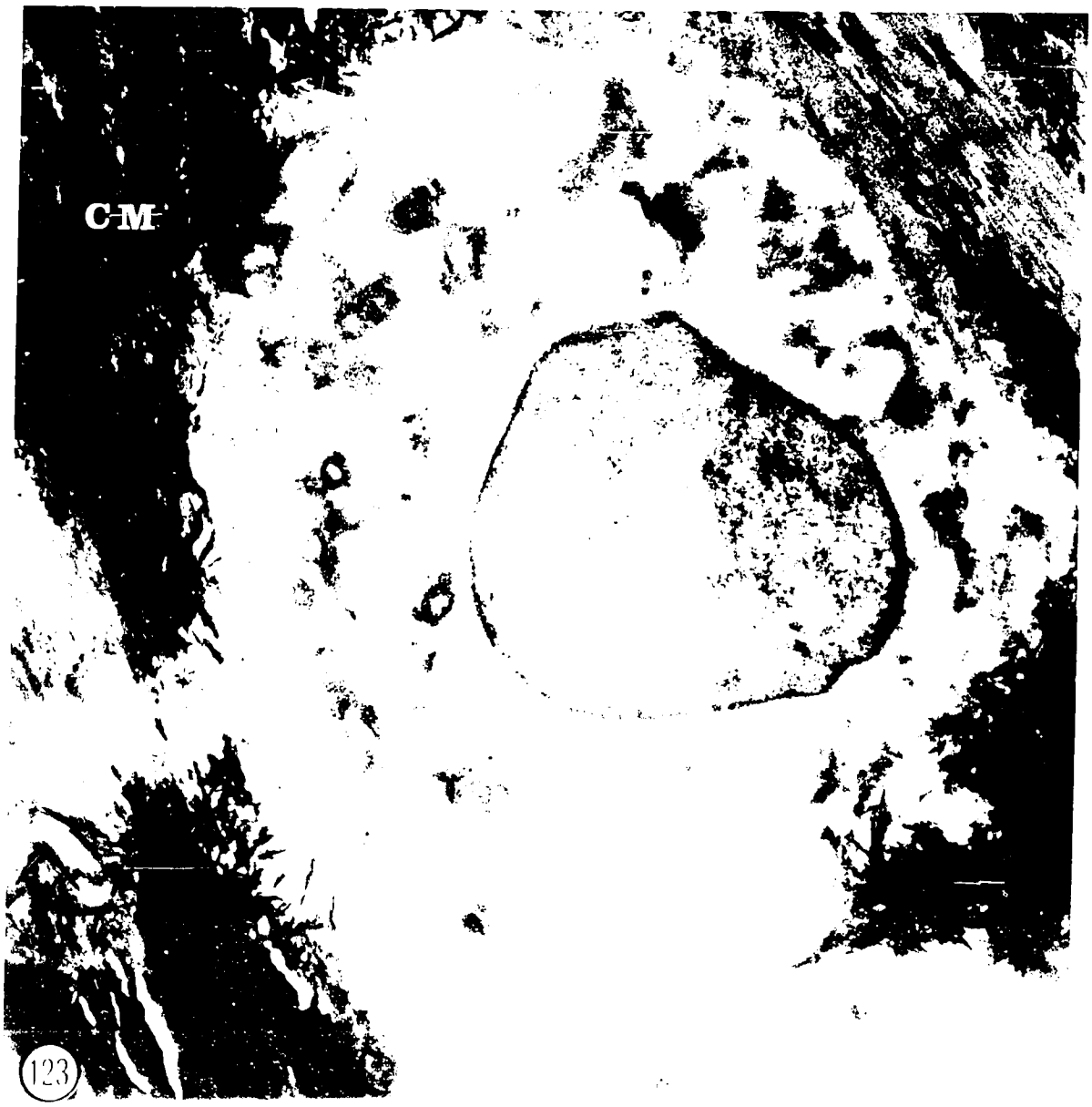


123

Single cilia were more frequently observed in fibroblasts of regenerating tendon from one week to three weeks after tenotomy. Several of them managed to reach the free surface or extracellular spaces. However, most appeared to be still within the Golgi zone of the fibroblasts (Figs. 125, 126, 127). The shaft of the cilium was separated from the cytoplasm by a ciliary vesicle (Figs. 125, 126, 127, 128). Single cilia have also been observed in fibroblasts of chick embryo dermis in cultures, but the number of these is not included in the table.

The most characteristic feature of cilia in various tissues was the presence of a centriole at the base, in addition to the basal body (Figs. 126, 128). This centriole was usually arranged at an angle to the basal body.

Only one cilium has been found in a chondrocyte (Fig. 129). The cilium appeared as a small solitary projection on the surface. Tubular structures could be seen in the cilium. The basal body and possibly a centriole could also be indentified. In addition, two satellites (Sorokin, 1968), one on each side, have been observed in the basal body.

In mucous metaplasia, (i.e., cheek pouch and vaginal epithelium and vitamin A treated chick embryo epidermis), single cilia were found mostly in the basal cells. In the control skin culture, single cilia have been observed in periderm (Fig. 130) and epidermis at various stages of differentiation.

It was, indeed, a great surprise to find a ciliated cell in the epithelium of hamster cheek pouch treated with vitamin A pellet.

Figure 124. A single cilium (arrow) from metaplastic urinary bladder epithelium of a rat with vitamin A deficiency. The cilium is still enclosed in the cytoplasm. A basal body can be identified at the base of the cilium. Vestopal.

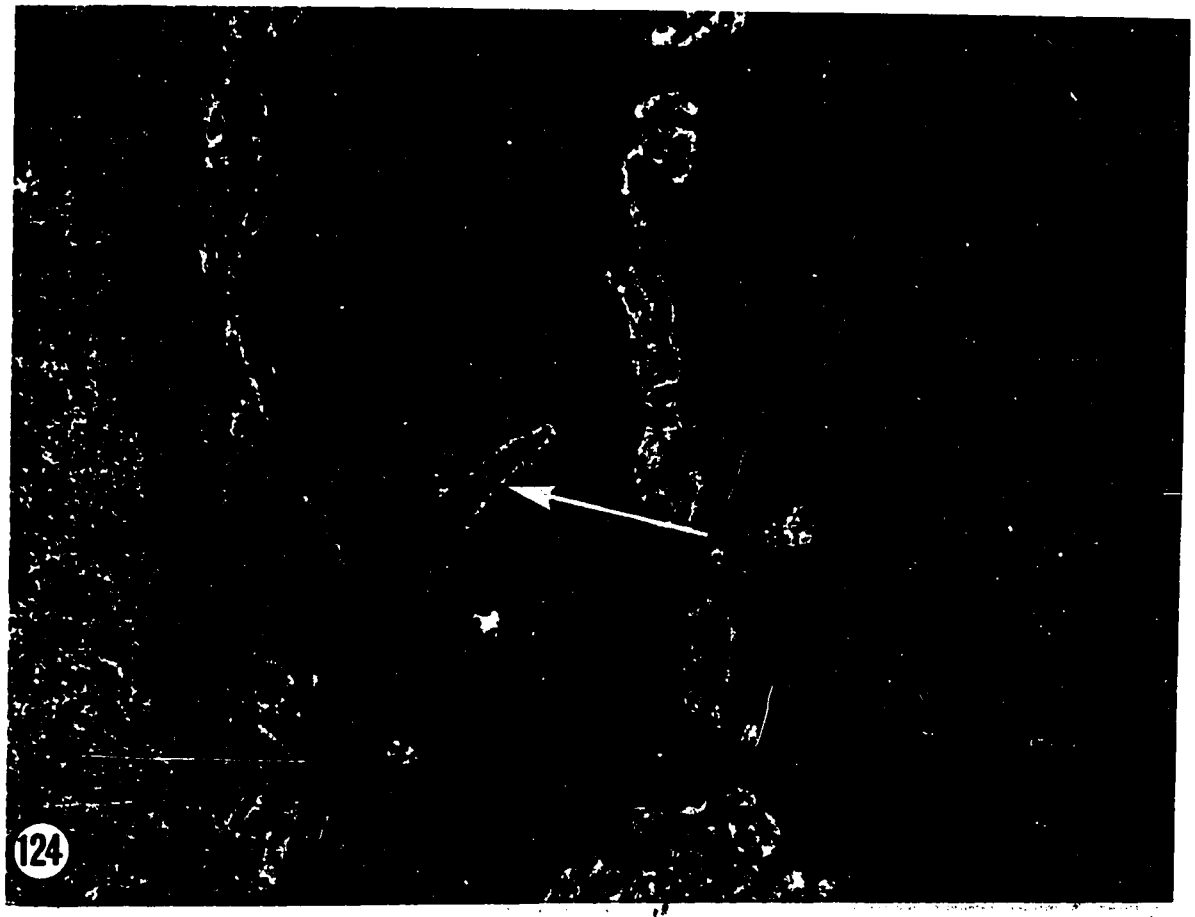
X 13,600

Figure 125. A developing cilium from a fibroblast of regenerating tendon. A basal body (Bb) and the ciliary vesicle (cv) can clearly be seen. Durcupan.

X 50,820

Figure 126. A developing cilium from a fibroblast of regenerating tendon, the ciliary vesicle (cv) ensheathing the growing shaft of the cilium. Microtubules can be seen extending from the basal body (Bb) into the shaft of the cilium. A centriole (c) which is arranged perpendicular to the basal body of the cilium is also shown. Durcupan.

X 43,830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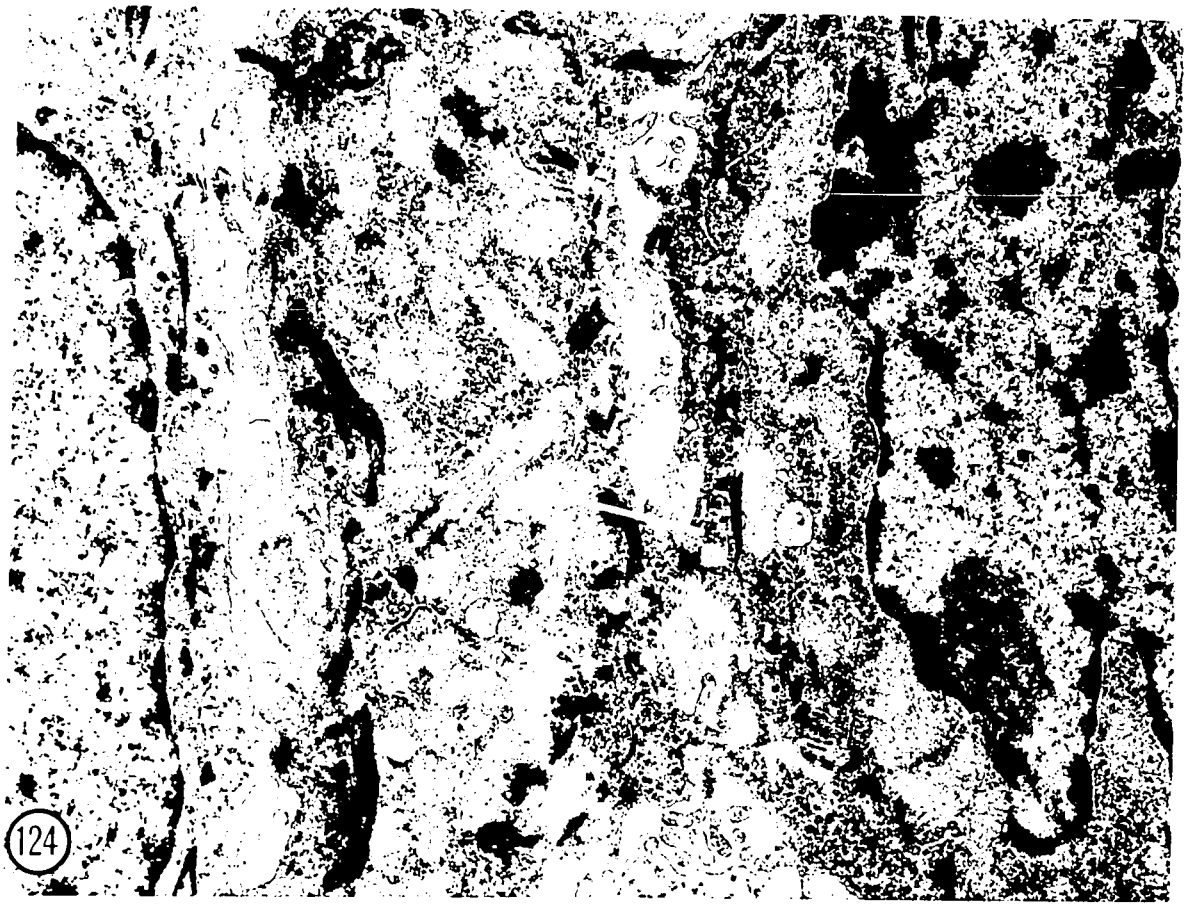
125


126



124

125

126

Figure 127. A developing cilium from a fibroblast of regenerating tendon. The shaft of the cilium is separated by the ciliary vesicle (cv) from the cytoplasm. The ciliary vesicle appears much larger than in the previous picture. Durcupan.

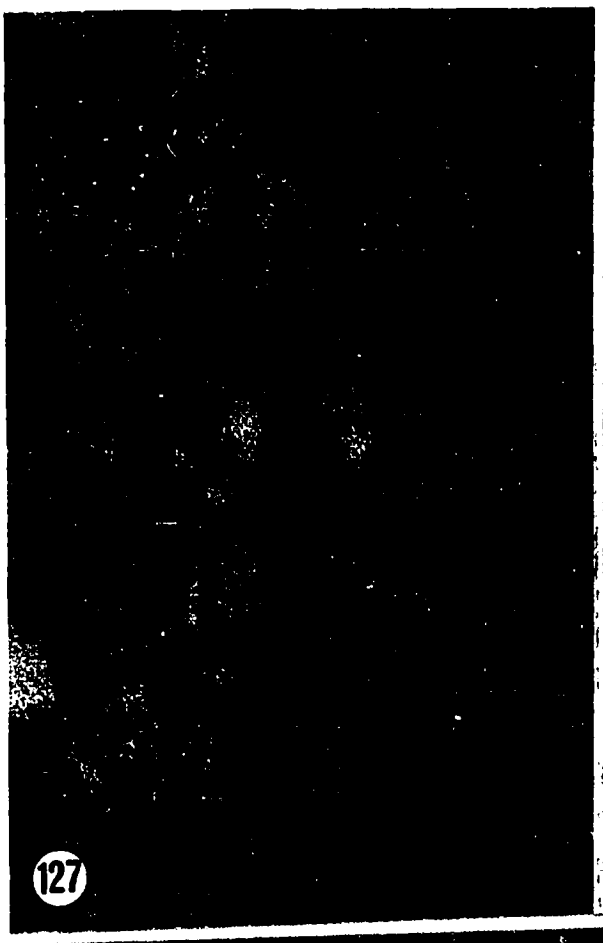
X 50,820

Figure 128. An oblique section through a cilium from a fibroblast of the chick embryo dermis in culture. The cilium has reached the extracellular space (Ecs) and the ciliary vesicle has fused with the plasma membrane of the fibroblast. A basal body (Bb) and a centriol can be seen. Durcupan.

X 73,600

Figure 129. An oblique section through a cilium of a metaplastic chondrocyte. The basal body and possible rootlet (►) can be identified. Epon.

X 110,000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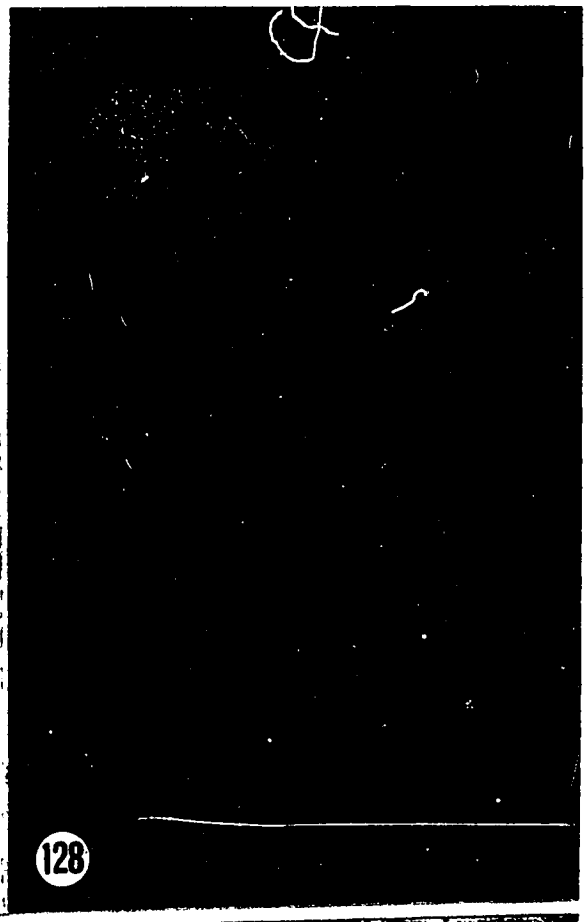
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129
L

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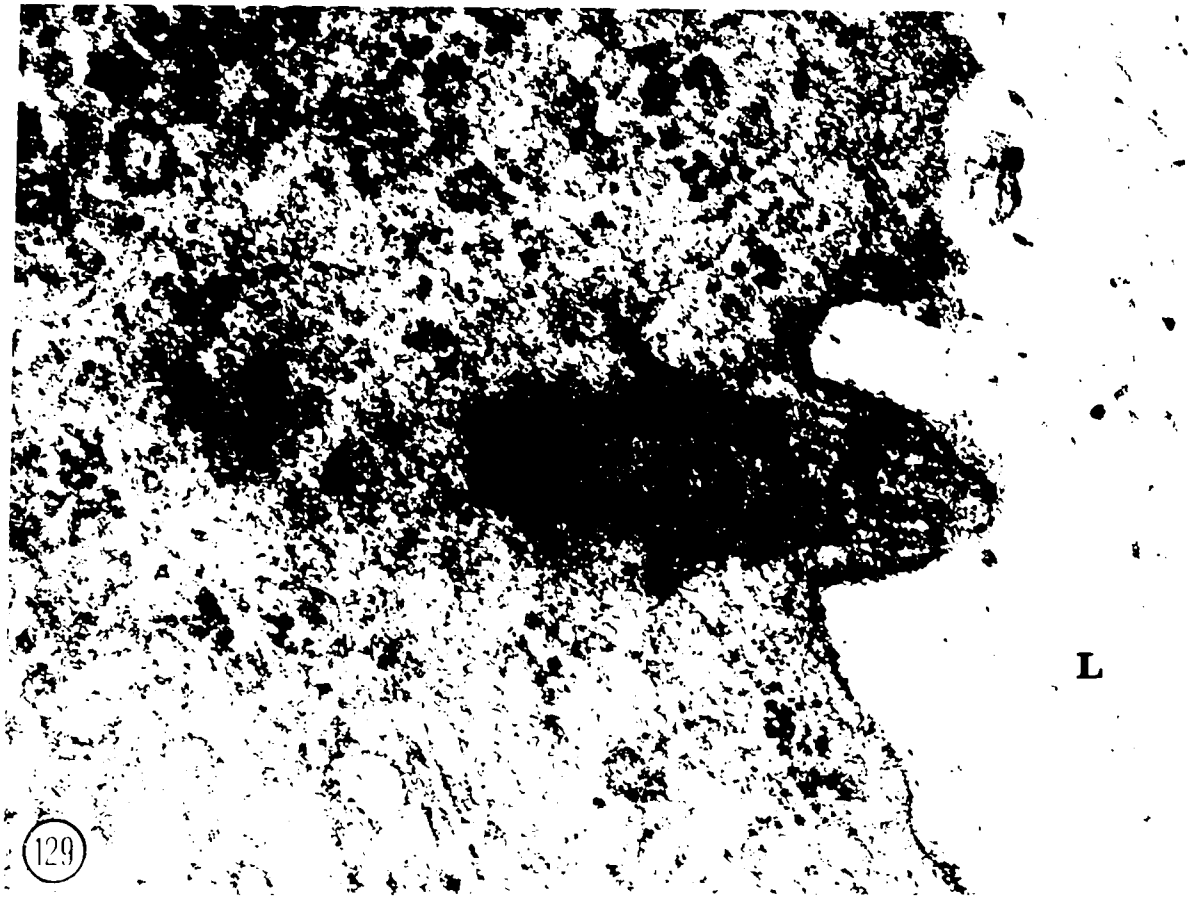
128



129

This ciliated cell (Fig. 131) was comparable to the typical cells in the trachea. Several long, well-developed cilia (Fig. 132), together with many long slender microvilli (some branched), lined the free surface of the cell. A basal body was found accompanying each of the cilia.

(a) Structure of the cilia.

It was not possible to ascertain the structure of all the single cilia observed here because serial sections were not cut. In general, the cilia observed here had nine sets of doublet tubules in the periphery (Fig. 133). The microtubule pairs were connected by dense material to the ciliary membrane. The two cental tubules were not observed in the few cross sectioned pictures.

(b) Development of cilia.

The development of cilia has been studied very extensively in various tissues (Sorokin, 1962, 1968; Kalnins and Porter, 1969). In the present study images evidently representing stages in the development of cilia from the centriole were encountered (Figs. 125, 126, 127, 128). The following is an interpretation of these images: At first, a solitary vesicle appears at one end of a centriole (Fig. 125). The ciliary bud appears to grow out from the same end of the centriole and invaginates the sac of this ciliary vesicle, which then becomes the temporary ciliary sheath (Fig. 126). Later, the bud lengthens into a shaft, while the sheath enlarges to contain it (Fig. 127). Shaft and sheath reach the surface of the cell, where the sheath fuses with the plasma membrane (Fig. 128).

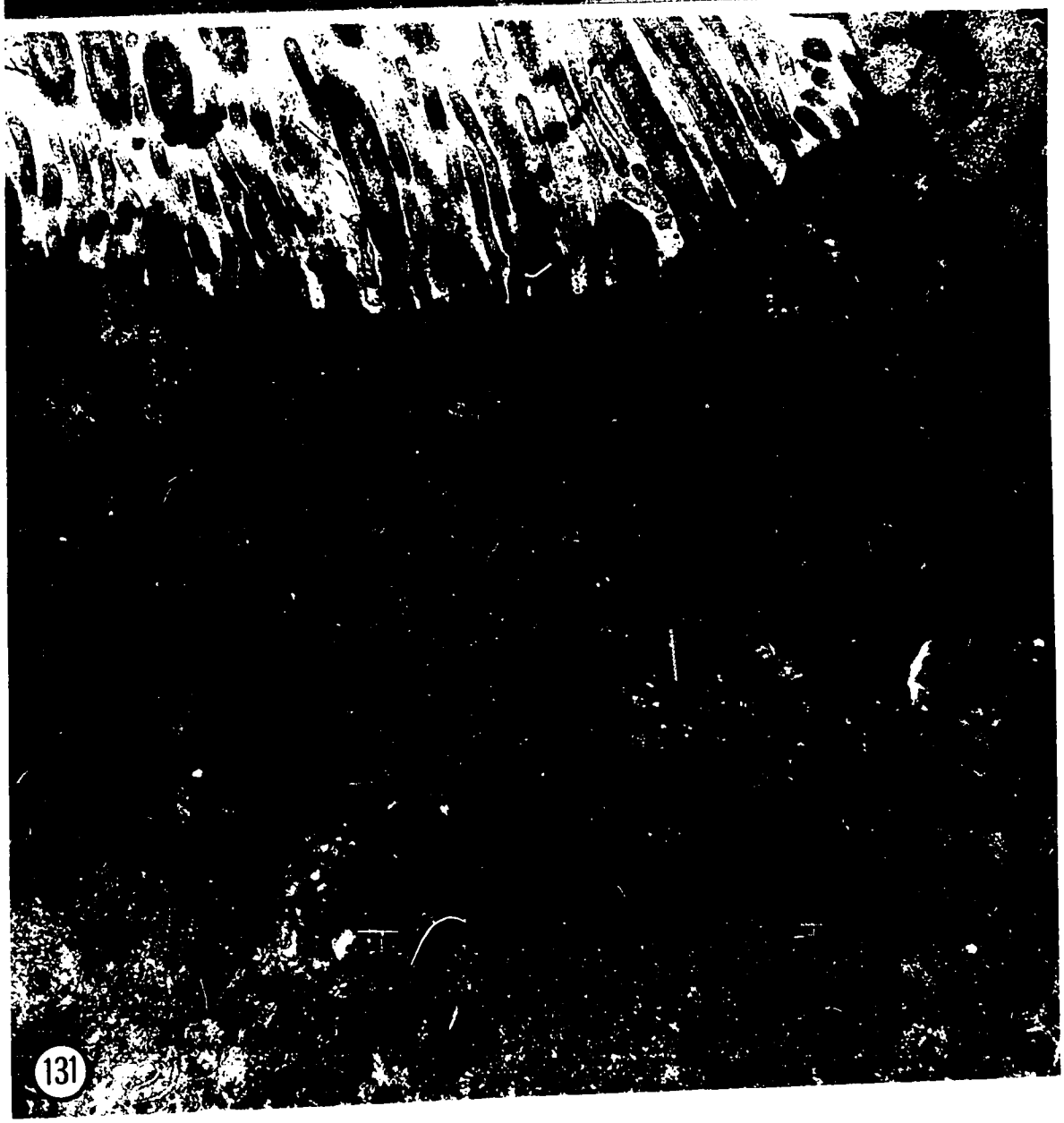
Figure 130. A cilium of peridermal cell of thick embryo skin in culture. Epon.

X 87,500

Figure 131. A ciliated cell from hamster cheek pouch treated with vitamin A pellet for 25 days. Typical cilia are found on the free edge of the cells. Microvilli, some of them branched, are present between cilia. This ciliated cell is quite comparable to those in the trachea. Epon.

X 23,310

130



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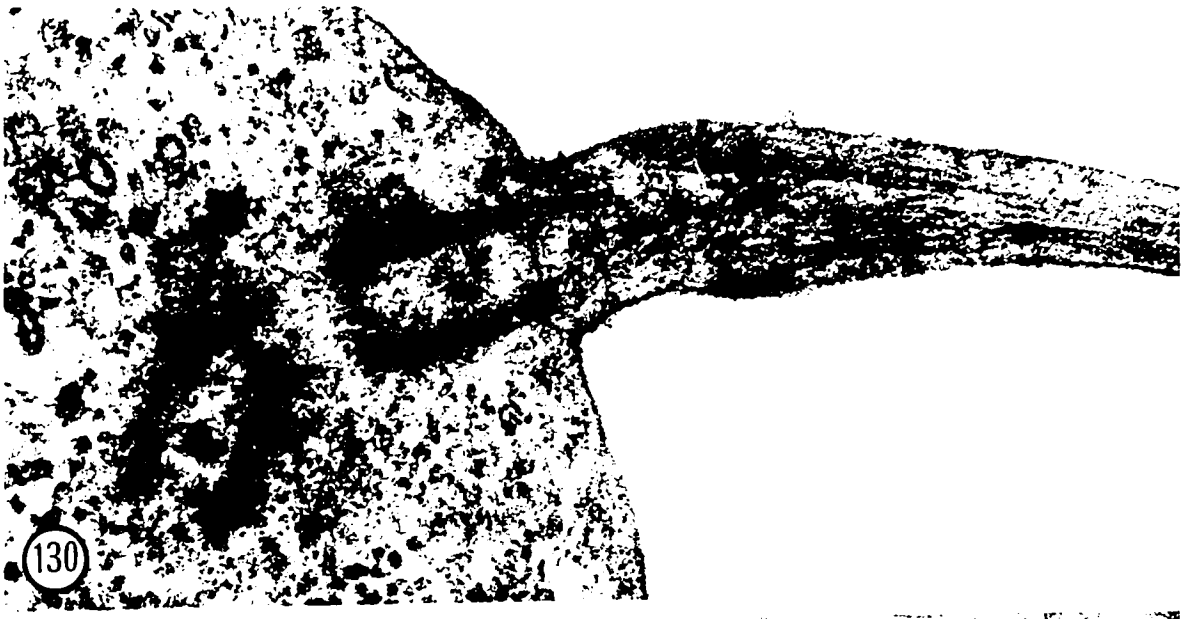

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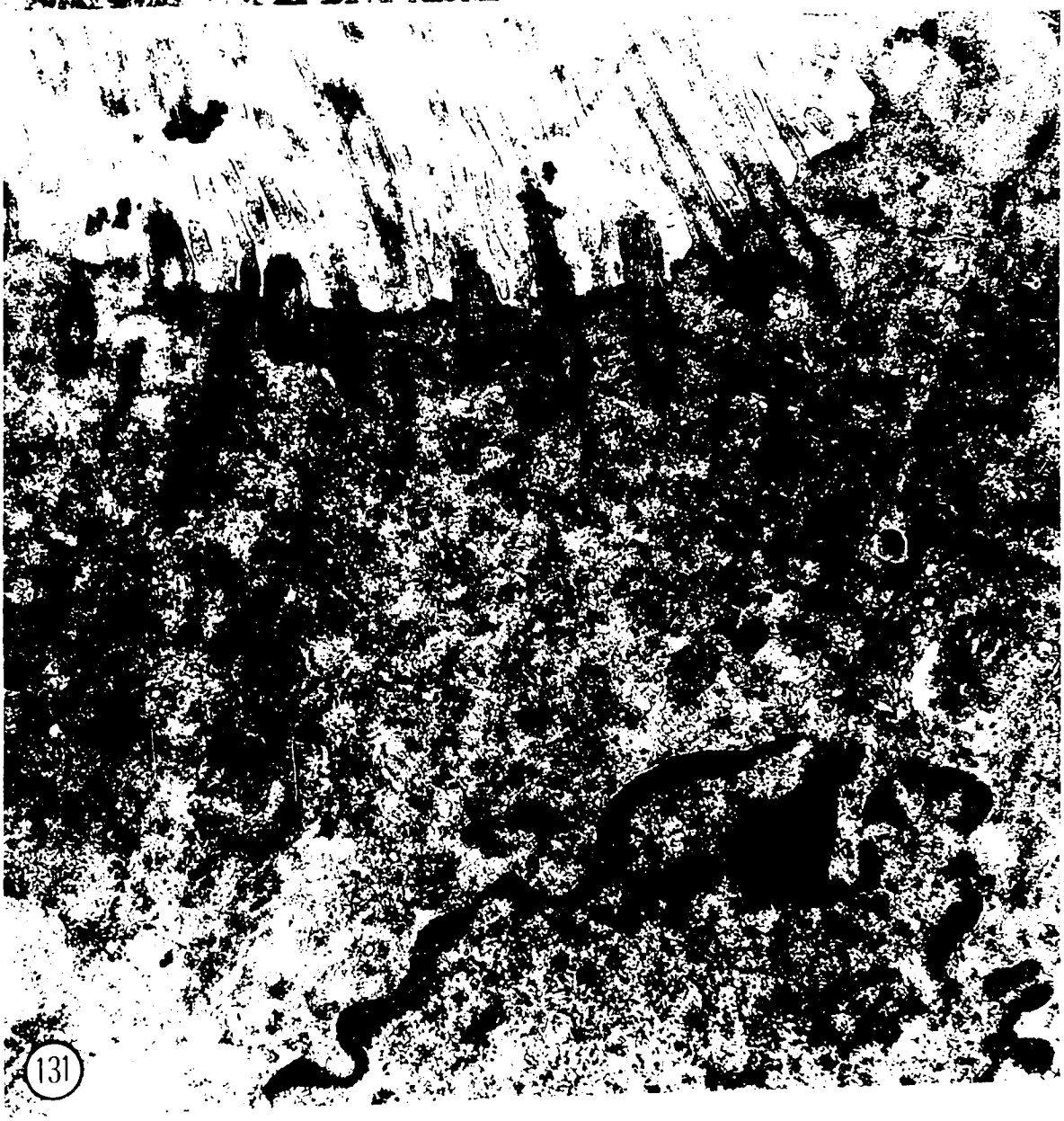


131

Figure 132. A high magnification picture showing several cilia and microvilli of the ciliated cell of Fig. 131. The microtubules in the cilia can clearly be seen. Epon.

X 54,400

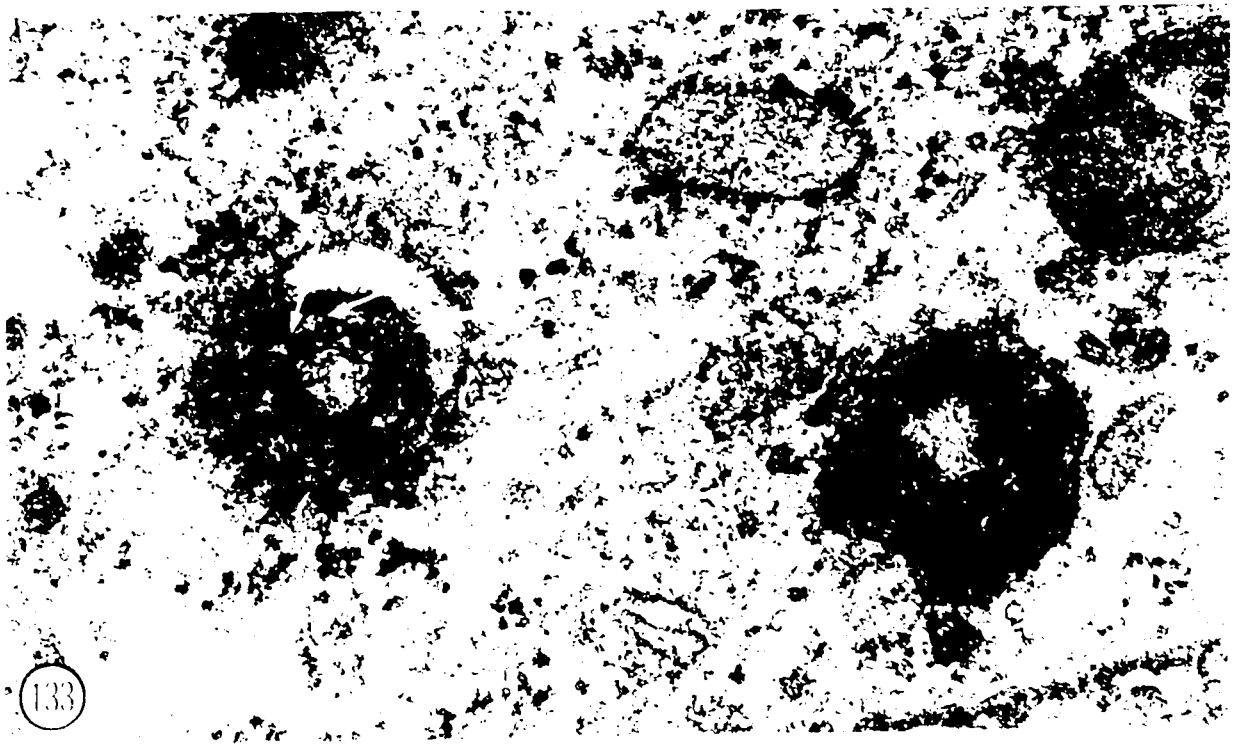
Figure 133. Electron micrograph showing a cilium (arrow) cut transversely near its base. Some of the microtubule doublets can clearly be seen. The microtubule doublets appear to be linked to ciliary membrane by dense material (small arrows). Epon.

X 100,000


132


133


132


133

V. DISCUSSION

1. Actions of Vitamin A

The mechanism by which the vitamin A acts on cells and in relation to metaplasia is not known although extensive studies have been made in the past both at the cellular and the subcellular levels. The following is an attempt to review some of the knowledge about the action of this vitamin.

It is known that in vitamin A deficiency, animals suffer from a number of symptoms including impaired vision, retarded growth, increased susceptibility to infection, blindness and ultimately death (see reviews, Moore, 1957; Jolly, 1967a,b). Recently it has been reported that the lack of appetite leading to lowered food intake might be due to the disruption of taste buds (Olson, 1969b). The sex organs in both male and female are deranged histologically and adrenal glands become atrophic. In addition a number of mucosal surfaces are found to undergo squamous metaplasia (Wolbach and Howe, 1925) including trachea, urinary bladder, ducts of salivary and lacrimal glands, prostate glands and cornea of the eye. The glandular secretion is greatly impaired. In an *in vitro* study Lasnitzki (1962) observed squamous metaplasia of prostate gland of young adult mice when cultured in chemically defined medium without added vitamin A.

Morphologically, in addition to what is reported in this

investigation, it has previously been demonstrated by Hicks (1968), Ghidoni and Campbell (1969) in urinary bladder, Hayes, McCombs and Faberty (1970) in parotid duct and Beith (1970) in cornea that squamous change occurs with vitamin A deficiency.

At the subcellular level attempts have been made to find the defects (or changes) associated with physiological changes initiated by vitamin A deficiency. Since under vitamin A deficiency mucosal surfaces undergo squamous metaplasia and glandular secretion is impaired, the first impression is that vitamin A might have some relation to mucopolysaccharide synthesis. Wolf and Varandani (1960), in a study of the incorporation of S^{35}- sulphate into mucopolysaccharides of colon homogenates, found that the incorporation in preparations of the vitamin A deficient rats was only half the normal. When retinol, retinal, or retenoic acid (vitamin A) was added the incorporation was restored to normal. Wolf and his associates (Wolf, Varandani and Johnson, 1961) thought that this change was due to inactivation of an enzyme fraction of the colon mucosa. However, when retinol was added to this fraction it showed little increase in S^{35} up-take. Instead, an increase was observed with a labelled lipid metabolite from retinoic acid (Wolf, Barga and Sundaresan, 1963). Thus, it would appear that the effect of vitamin A on mucopolysaccharides might be exerted indirectly through the enzyme system. However, Pasternak, Humphries and Pirie (1963) were not able to confirm Wolf's results.

Wolf (1962) also speculated that vitamin A releases an enzyme (ATP sulfurylase) (or enzymes) necessary for the biosynthesis of mucopolysaccharide from the particle fraction sedementing at

20,000 g, by altering the permeability of the membrane of the particles.

In addition to ATP sulfurylase, Olson (1969b) has recently implicated many other enzymes, including Δ^5, 3β-Hydroxyl steroid dehydrogenase and 11β steroid hydroxylase (for steroid synthesis), and has stated that oxidative phosphorylation may be affected.

DeLuca, Manett, Madsen and Olson (1963), finding that vitamin A deficiency in rats raises the rate of oxidation of most substrates of the tricarboxylic acid cycle by liver homogenates, were led to suggest that vitamin A acts on a system common to all the tricarboxylic acid cycle substrates, such as electron transport oxidative phosphorylation or mitochondrial structure.

Roels, Trout and Guha (1964) demonstrated that the stability of liver lysosomes of vitamin A deficient rats was greatly impaired. They found that in the cytoplasmic extract, mitochondrial-lysosome rich fraction, and microsome fraction, the "free" lysosomal enzyme was two to three times higher than in controls, while in the supernatant fraction, it was more than three times higher than the control.

However, the relationship of these findings to the problems of squamous metaplasia in epithelial surfaces is not known. It is obvious that more studies are needed before any solid answer to this question can be provided.

The other line of investigation is through the study of the response of tissues, cells or subcellular organelles to excess of vitamin A. The relationship between mucous metaplasia and hypervitaminosis A is well known, as has been reviewed in the section, Historical Review.

In *in vitro* studies Fell and Mellanby (1950, 1952) found that when vitamin A was added to the foetal bone in culture the normal growth of bone was stopped. Fell, Mellanby and Pelc (1954) showed that the addition of excess vitamin A to tissue culture medium caused dissolution of the mucoprotein or cartilage.

In an *in vivo* study it has been shown (Thomas, McCluskey, Potter and Weisman, 1960) that administration of large amounts of vitamin A to rabbits resulted in depletion of the cartilage matrix. Rabbits after hypervitaminotic A treatment had collapsed ears. A similar effect could be obtained by injection of papain (a vegetable protease). They, therefore, suggested that the changes in cartilage might result from activation of proteolytic enzymes produced by hypervitaminosis A. Fell and Thomas (1960) later observed similar results in embryonic cartilage in organ cultures with papain and with vitamin A.

Dingle, Lucy and Fell (1961) noticed a considerably increased acid soluble nitrogen which they thought to be due to proteolytic enzymes in the culture medium. When liver lysosomes were incubated with addition of vitamin A, protease was released from the lysosomes (Dingle, 1961). Fell and Dingle in 1963 further demonstrated that acid protease, released from cartilage in organ culture (with retinol), was similar to that of the protease from lysosomes. Bassett and Packer (1965), studying the effect of vitamin A on pure lysosome (mitochondria-free), found that the increased release of acid phosphatase from the lysosomal fraction was accompanied by swelling and disruption of the particles. The site of action was probably on the lysosomal membrane. The effect of vitamin A on bone in

culture could be greatly retarded by adding hydrocortisone to the medium (Fell and Thomas, 1961). This hormone has been shown to stabilize lysosomal and other membrane systems (de Duve, Wattiaux and Wibo, 1962).

Lysosomal membranes are not the only membranes acted upon by vitamin A. Dingle and Lucy (1962) and Kinsky (1963) have shown that retinol caused the lysis of the membrane of red blood cells. The initial action of vitamin A on erythrocytes is believed to be a penetration and expansion of the cell membrane (Glauert, Daniel, Lucy and Dingle, 1963). When retinol was added to fibroblasts in normal saline the plasma membrane quickly disintegrated (Dingle, Glauert, Daniel and Lucy, 1962; Lucy, 1964). In a subsequent study, Daniel, Dingle, Glauert and Lucy (1966) observed some disorganization of the mitochondrial cristae, a diminution of the cisternae of the RER, and an increase in the amount of smooth membrane and free ribosomes. They also observed a rapid decline in respiratory activity of the cells. They believed that the primary action of excess of retinol was to cause alteration in the membranes of the cells and that these alterations in turn affected the functions of the mitochondria and endoplasmic reticulum.

However, the question of the relationship between hypervitaminosis and mucous metaplasia still exists. Although vitamin A does stimulate the synthesis of RNA in the colon of deficient rats (Zachman, 1967) and DNA in epidermis (Christophers and Braun-Falco, 1968), the effect, according to Olson (1969a,b), might be indirect. It could be this indirect effect that results in a change in phenotypic expression.

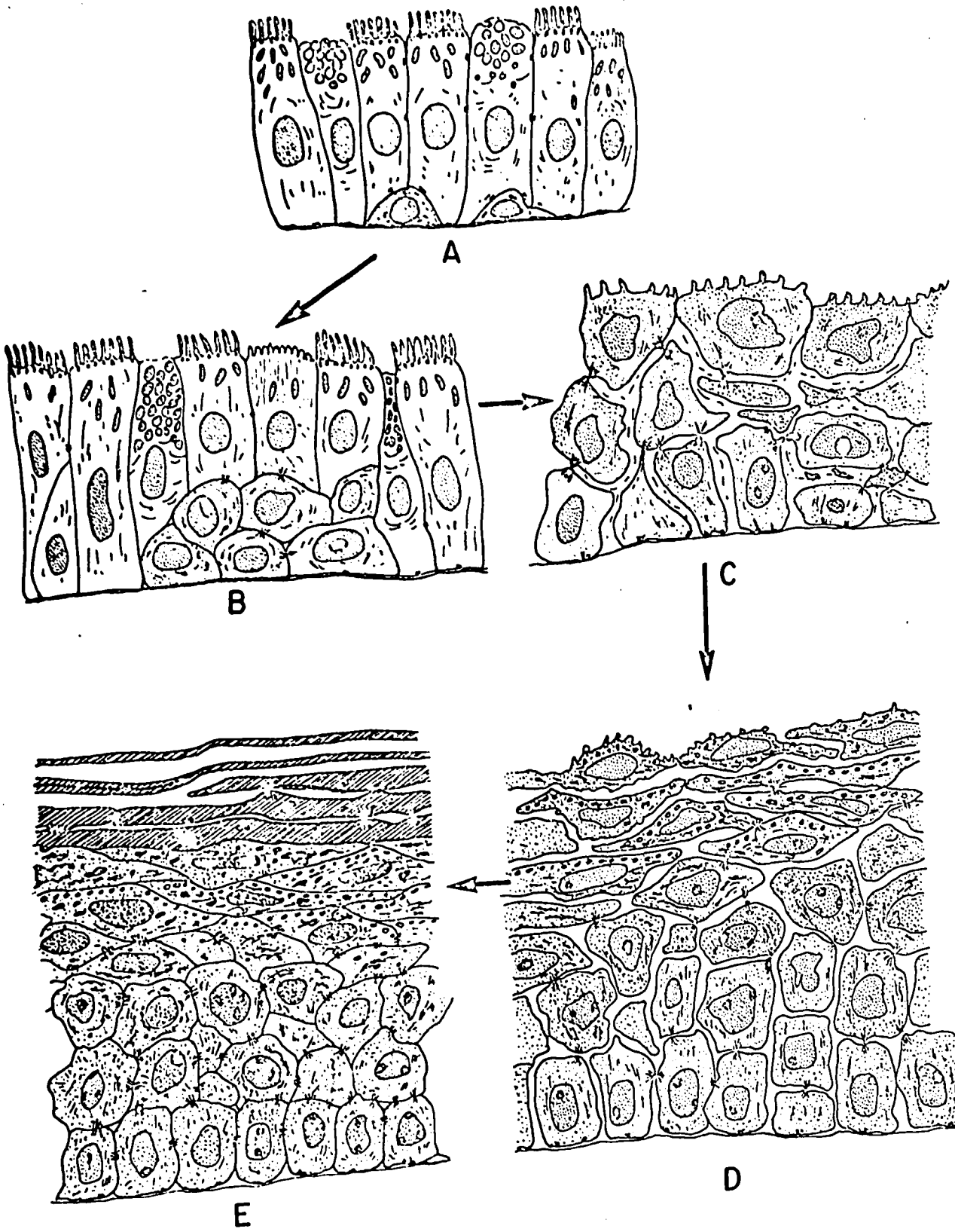
2. Epidermoid Metaplasia in vivo.

The results of the present study seem to indicate that the metaplastic transformation of bladder and tracheal epithelium to cornified epithelium under vitamin A deficiency is closely related to hyperplasia.

From a study of the pathogenesis of carcinogen-induced tumors of the tracheal epithelium, Kotin, Courington and Falk (1966) concluded that the carcinogen acted on the basal cells. Basal cell hyperplasia was followed by squamous metaplasia, and ultimately by neoplastic growth. Although a very different method has been used to produce metaplastic change in the present investigation, the results indicate that, in this case also, metaplasia is preceded by basal cell hyperplasia.

The results are summarized in Chart 3. Hyperplastic clusters of cells are at first localized, with several foci in one animal. The foci then spread over a larger area, and many mitotic cells are seen during this period. The cell layers superficial to these hyperplastic basal cells, consisting of somewhat altered respiratory epithelium, are undercut and desquamate. The loosely associated hyperplastic cells differentiate into flatter cells towards the surface, and these begin to show such specific features as membrane-coating granules, keratohyalin granules, desmosomes, and keratin fibrils. The development of a fully cornified surface layer concludes the process of differentiation. In short, the stages are those of hyperplasia of basal cells, stratification and cornification (Chart 3).

Chart 3. Schematic representation of the sequence of steps leading toward a squamous metaplasia in the rat tracheal epithelium in vitamin A deficiency. A, Normal tracheal epithelium; B, Initial stage of hyperplasia; C and D, Stratification; E, Cornification.


A
B
C
D
E

It should be noted that the development of a cornified layer is only achieved by differentiation beginning in the basal cells, which have the capability to divide and the flexibility for differentiation. Whatever the stimulus that produces a change in the final differentiated cells, it might be expected to act on the basal cells. The fact that the differentiated (ciliated and goblet) cells showed no tendency to keratinization would indicate that their potential for differentiation into keratinized cells was absent or not expressed under the conditions of these experiments.

Hyperplasia of bladder epithelium was characterized by an increase in thickness of the stratum intermedium. Although mitotic figures were observed frequently in the basal cells, nests of hyperplastic cells, as observed in the tracheal epithelium have not been found in the bladder. The reason that nests of basal cells were not recognized might be that the basal and intermediate cells did not have great cytological differences. As soon as the division was completed the resulting cell was presumably being pushed up and could not be distinguished from intermediate cells.

As in the trachea, layers of normal bladder epithelium were sometimes observed lying above the keratinized cells. The interpretation is that when groups of hyperplastic cells have been formed through proliferation of basal cells some functional changes occurred in cells of the basal region that initiated a new line of differentiation characterized by keratin fibril synthesis. The deep hyperplastic and keratinizing epithelium then expanded and pushed up the surface hyperplastic cells. The "old" hyperplastic bladder

epithelium overlying it was then desquamated with or without cytological changes. Finally, a stratum corneum was differentiated, and completed the metaplastic transformation. In short, the stages of differentiation were hyperplasia, followed by semi- and complete cornification, but the extensive stratification seen in metaplasia of trachea was not a feature.

The modified fusiform vesicles (type I) found in semi-cornified bladder epithelium, like the normal vesicles of the squamous cells, were bounded by asymmetric unit membrane, as reported by various investigators (Porter et al., 1967; Hicks, 1965; Koss, 1969) and in the present investigation. Unlike the normal fusiform vesicles, these vesicles were filled with electron dense material. Its chemical nature and its origin are not known. It is unlikely that this material could be added after the vesicles had been produced. The vesicles were presumably closed by that time. It would appear more likely that the material was added when the vesicles were still "open", that is, before they were pinched off from the Golgi cisternae. The type II vesicles appear to be homologous to the type I, but more uniform in size, and their AUM appeared less angular, and therefore, perhaps less rigid. They resembled the granules of the first type in having AUM, but the MCG in shape, size and location.

It is believed that the continuum of morphology of vesicles (or granules) reflected the functional changes in the superficial cells of the semi-cornified bladder epithelium. The GA of the superficial cells, while retaining the capability of synthesizing AUM,

also produced MCG-like matrix. When the former is the dominant activity, type I vesicles develop. Alternatively, the type II or MCG-like vesicles are produced when the stimulus towards keratinization increases.

It is believed that the superficial cells were not eventually cornified, and hence were not part of the final cornified epithelium. This epithelium was later desquamated without cornification. However, certain changes or modification of function and structure in the cells did occur, as seen by the appearance of various types of vesicles and the KH granules in bladder or loss of cilia, for example, in trachea.

The structure of the metaplastic (cornified) bladder epithelium was quite similar to that of the cornified tracheal epithelium. The MCG of cornified bladder epithelium were larger than those of the trachea. The former had an average width of approximately 150 mμ, which was the same as that of epidermis, while the latter were only about 100 mμ.

Fibrillary material in various layers of cells as reported here was much more abundant than in the metaplastic tracheal epithelium, the cytoplasm of the cells of stratum spinosum particularly, being packed with keratin fibrils.

The KH granules of the cornified bladder were more numerous and larger than in the trachea.

Tight junctions were not found in tracheal metaplasia, but quite often seen in the bladder.

Desmosomes were bigger, and the modified membranes of the bladder epithelium were thicker (200A°) than those in trachea (110A°).

Thickening of the plasma membrane of bladder was recognized two-three layers beneath the horny cells in some regions. It was not apparent in the trachea until the cell just beneath the stratum corneum.

In comparing these two cornified epithelia under the same conditions, one gets the impression that the metaplastic bladder resembles epidermis more than did the cornified tracheal epithelium, in respect to cytodifferentiation. The reason for this is not clear, in view of the same experimental conditions which existed. Hicks (1966) believed that the transitional epithelium lining the rat urinary bladder might be regarded as a keratinizing epithelium in which cornification did not normally occur. If this were true, it is understandable that the metaplastic bladder was better developed or differentiated than metaplastic trachea under conditions of vitamin A deficiency. The former has the intrinsic keratin-synthesizing "machinery" which is presumably lacking, or developed to a much lesser extent in the normal tracheal epithelium.

3. Keratohyalin Granules, Membrane-Coating Granules and Keratinization.

The keratinization of metaplastic tracheal and urinary bladder epithelia is strikingly similar to the process described in epidermis and oral mucosa by Matoltsy and Parakkal (1965), consisting of the formation of filaments or keratin fibrils, MCG, KH granules and thickened plasma membrane of the horny cells. The presence of large numbers of free ribosomes and polysomes in

the basal cells and prickle cells is obviously related to the appearance of keratin filaments. In the further course of differentiation the filaments became grouped into bundles, forming keratin fibrils.

KH granules were another prominent feature in both of these metaplastic epithelia and also in the non-cornifying superficial cells of the semi-cornified bladder epithelium. Areas of non-cornified granular hyperplasia, characterized by the presence of KH granules in the squamous cells, were reported by Hicks (1968) in the rat bladder in vitamin A deficiency. In this investigation no such areas were found. However, KH granules were observed in some superficial cells of semi-cornified areas, together with various types of modified vesicles. It appears, as judged from the variety of changes observed during this investigation and those reported by Hicks (1968), that the formation of KH granules is not necessarily associated with keratinization.

The close association of KH granules with ribosomes has been reported in skin and oral mucous membrane (Brody, 1964; Montagna, 1962; Rhodin and Reith, 1962; Rogers, 1964), and in bladder epithelium (Hicks, 1968; Ghidoni and Campbell, 1969). It was Hicks who first suggested that the KH granules in hyperplastic or in cornified areas of the bladder are largely derived from the condensation of morphological altered ribosomes. In this investigation a close association of KH granules with ribosomes and no spacial relationship to the keratin fibrils were observed. These observations tend to support the view of Hicks. But, whether or not KH granules are made up only

of ribosomes is rather questionable. They appeared to have two morphologically distinct portions, as seen in the cornified bladder epithelium. Most of the KH granules (type 1) had a granular substructure with a particle size similar to that of ribosomes. Other granules were very dense (type 2) and did not reveal any internal structure. The dense components were either isolated in the cytoplasm or the nucleus, or located peripheral to the main granular mass of the KH granules.

The origin of these KH granules is not clear. Hicks, referring to the change of granularity in the vitamin A deficient rat bladder, stated that "the dense amorphous material.....is derived from aggregates of ribosomes, which becomes progressively more condensed until their morphology changes from granular to an amorphous form.....". If this is the case one would expect this dense material to be the central core of the KH granules, which was the case in the metaplastic tracheal epithelium and in the picture of Hicks (1968), but rarely in the present study of the urinary bladder.

Recently, Jessen (1970) has reported two types of KH granules in the rat tongue epithelium. He called them single and composite granules. Single granules were smaller, dense, homogenous, and were comparable to the type 2 KH granules observed in the urinary bladder. Composite granules were made of single granules embedded in granular matrix material, and were found only in the cytoplasm. The composite granules are probably similar to the combination of dense granules (type 2) and ribosomes aggregates (type 1). Jessen (1970) also re-

ported that the matrix of composite granules is protein in nature and digestible with trypsin. In the present investigation it was not possible to carry out a similar test because the Vestopal used for embedding is too impermeable for this cytochemical test.

There are two possible explanations for the difference in morphology of KH granules: (1) They are different in chemical nature or (2) they are of different origin, i.e., the granular part is of cytoplasmic and the dense part is of nuclear origin.

It was earlier believed that KH granules represent a stage in the synthesis of keratin fibrils. Mercer (1961) on the basis of chemical studies, postulated an alternative pathway for keratinogenesis in which sulphur-poor filament precursors temporarily accumulated in an amorphous form as KH granules. Brody (1959), from a study of the fine structure and staining characteristics of KH granules believed that the material in the granules was the source of the interfilamentous matrix of the horny cells. Because KH granules are a common feature of mammalian epidermis it was believed that these granules must have active roles in keratinization. The present observation, and that of Hicks (1968), show that KH granules may exist in epithelial cells showing no sign of cornification. In snakes (Roth and Jones, 1967) and frogs (Farquhar and Palade, 1965) KH granules do not appear in epidermal cells, while in psoriatic parakeratosis the formation of keratin may proceed independently of the KH granules (Brody, 1962).

As shown in the present investigation, the MCG in the metaplastic tracheal and bladder epithelia are similar in structure

and distribution to those of normal epidermis (Farbman, 1964; Frei and Sheldon, 1961; Frithiof and Wersall, 1965; Matoltsy, 1966; Matoltsy and Parakkal, 1965; Odland, 1960). In normal skin and other keratinizing epithelia MCG deposit their laminated contents into the extracellular space. In the present investigation fusion of MCG with plasma membrane has been observed in tracheal, though not in urinary bladder metaplasia. The laminated content of MCG has been observed in intercellular spaces in both cases. It is believed that the MCG granules were discharged into intercellular spaces in both metaplastic epithelia.

Some controversy is associated with the significance of these granules. Matoltsy and Parakkal (1967) believed that MCG contributed to the thickened plasma membrane of the horny cells. Hashimoto, Gross, Nelson and Lever (1966) believed that these granules contained polysaccharide, and this evidently would imply that the MCG had a role in binding together the horny layers. Wolff and Holubar (1967) and Wolff and Schreiner (1970a) have shown that MCG in skin contained acid phosphatase. The activity of this enzyme could be demonstrated in MCG within epithelial cells as well as in the MCG remnants in the extracellular spaces. This has recently been confirmed by Weinstock and Wilram (1970) in the study of mouse tongue epithelium. It would seem that the MCG may be just another type of lysosome with a function in desquamation of horny cells, including horny cells of metaplastic tracheal and bladder epithelia.

The sequential events leading to the formation of horny

cells were similar in metaplastic tracheal and bladder epithelia and mammalian epidermis in respect of the formation of thickened cell membrane and decomposition of major organelles (Lavker and Matoltsy, 1970). The plasma membrane of keratinocytes of the bladder increased from 70 Å° to about 180 Å° in granular cells just beneath the horny layer. An average membrane thickness of 200 Å° (though much thinner in the case of trachea) was observed in the horny cells.

The desmosomes of the epidermis undergo structural modification at the junction of stratum granulosum and corneum. In these investigations a similar modification was observed best in granular cells of bladder epithelium several layers beneath the horny cells. This might be due to the relatively early thickening of the membrane of the bladder granular cells compared to those of the epidermis.

Matoltsy and Parakkal (1965) suggested that the deposition of the internal content of the granules contributes to thickening of the plasma membrane of cornified cells. On the other hand, Bonneville et al. (1968) and Farbman (1966) believed that the granules contributed to the intercellular material, but that the thickening of the plasma membrane of the cornified cells arose intracellularly by some other mechanism. A definitive conclusion was not obtained about this phenomenon in the present study. At the desmosomes, the inner attachment plate, continuous with the thickened membrane, was separated from the intercellular desmosomal plate by a gap which was equal to the central lamella of the unit membrane.

This observation suggests that the thickening of the membrane

is due to an intracellular process, as reported by Bonneville et al. (1968), Farbman (1966) and Frithiof (1970), rather than to the contents of the MCG. This conclusion is consistent with the recent report by Fukuyama and Epstein (1969) that a cysteine-containing protein appears to be incorporated into the plasma membrane of cornifying cells.

The intercellular desmosomal disc, laminated material of MCG, and degradation products of the granular cells (Lavker and Matoltsy, 1970) might be incorporated into the intercellular plates of horny cells.

4. Cell Coats

A mucous coat has recently been reported in transitional epithelium of human bladder (Monis and Zambrano, 1968b). So far, no similar demonstration has been made in rat transitional epithelium. It is worth noting that the surface coat of normal surface squamous cells, as reported here, was clearly demonstrated by ruthenium red. The staining of the surface coat of rat transitional epithelium with ruthenium red is an indication of the presence of glycoprotein. Ito (1965) believed that mucous coat is indeed part of the plasmalemma. Sialic acid has been demonstrated in bladder epithelium of man (Manis and Dorfman, 1967).

It is of interest to note that some "intra-cytoplasmic" vesicles in sectioned material were stained by ruthenium red while the majority of them were not. Clearly, some must be in one way or another connected to the luminal surface. These vesicles (which

would show typical AUM, were they not "stained" by ruthenium red) have been observed by previous investigators (Porter et al., 1967; Hicks, 1965) to become filled following the injection of ferritin into the bladder lumen of the living animal. The positive reaction of ruthenium red with AUM of vesicles would indicate that it contained, among other things, glycoprotein with an acid group (Luft, 1965a,b). However, whether the glycoprotein is part of AUM, or on the surface of the AUM is not known. Further work is needed to clarify this.

As for the functional properties of the mucous coat, it seems likely that aside from creating a microenvironment for the cell, its chemical heterogeneity would indicate that cell coats have different functional properties according to cell types. The reason that ruthenium red did not stain the surfaces of other epithelial cells of the bladder is believed to be due to the presence of tight junctions and the great thickness of the surface squamous cells. Ruthenium red was presumably kept away from other surfaces of the epithelium. Occas ional staining of some cell surfaces, deep in the epithelium may be cited as evidence for this. Hicks (1966) believed that there exists a permeability barrier of the rat bladder epithelium.

5. Relationship Between Squamous Metaplasia and Carcinoma.

It has been noted in the Historical Review that the close relationship between squamous metaplasia and carcinoma has led many investigators to regard it as a precancerous lesion.

The present experiments, on the other hand, seem not to suggest a precancerous condition, at least from the standpoint of the morphological changes occurring in the period of these experiments. Thus, metaplastic trachea and urinary bladder bear a striking resemblance to normal epidermis. In agreement with the observations of Hicks (1968) and Ghidoni and Campbell (1969) the basement membrane was found to remain intact; no loss of cell polarity has been found, as might be expected in pre-malignant lesions (Svejda and Dolezel, 1964; Feit, Svejda and Hoffman, 1967).

Although the question of reversibility of metaplasia induced by vitamin A deficiency was not studied in the present experiments, other workers have found that complete recovery results when the vitamin A level is restored (Wolbach, 1937).

In spite of the fact that the metaplasia is reversible under normal circumstances, which would suggest that it is not pre-malignant, the possibility remains that the metaplastic cells might well be more susceptible to the induction of neoplasia by carcinogenic agents. In man the relationship of metaplasia to squamous carcinoma of lung (Auerbach et al., 1957, 1961, 1962a,b; Valentine, 1957) and urinary carcinoma (Kelalis et al., 1963; O'Flynn and Mullaney, 1967) suggests that whatever factors induce the cancers in these organs, they were more effective in the presence of metaplastic change.

6. Langerhans Cells

The considerable number of studies on fine structure of the Langerhans cell have provided a firm basis for its identification in electron micrographs (Breathnach, 1964, 1965; Breathnach and Wyllie, 1964; Birbeck, Breathnach and Everall, 1961; Sagebiel and Reed, 1968; Wolff, 1967; Zelickson, 1965, 1966; Zelickson and Hartman, 1961). The characteristic granules are not found in melanocytes so that it is now possible to differentiate these two cell types on this basis. On the other hand, although premelanosomes have never-been observed in the Langerhans cell (Prunieras, 1969) the presence of melanosomes has been reported (Zelickson, 1965).

It was proposed that Langerhans cells might represent worn out (effete) melanocytes (Masson, 1951). More recent studies, however, indicate that these are not effete cells, on the contrary, it has been concluded that they exhibit protein synthetic activity (Kiistala and Mustakallio, 1967), uptake of exogenous protein (Wolff and Schreiner, 1970b) and DNA synthesis (Giacometti and Montagner, 1967; Schellander and Wolff, 1967). In the present study both types of granules are associated with the Golgi zone, and for that reason they are likely to represent a product of cell synthesis. Mitosis has been seen in a Langerhans cell by Hashimoto and Tarnowski (1968). In the present material, also, the existence of microtubules radiating out from the vicinity of the centriole is consistent with the appearance of an immediately pre- or post-mitotic phase in this cell.

It was recently suggested by Zelickson (1967) that a melanoblast may be stimulated to produce either a melanocyte or a Langerhans cell, and that each of these then populates the epidermis. Against this view is the evidence that Langerhans cells are present in the skin of limb buds before the cells of the neural crest have migrated (Breathnach et al., 1968).

It seems unlikely that the invasion of bladder or tracheal epithelium by Langerhans cells could represent migration from nearby or remote epidermis or other stratified squamous epitheium. Similarly, in the osseous and pulmonary lesions of histiocytosis X (Tarnowski and Hashimoto, 1967; Wolff and Sollerender, 1969) the origin of Langerhans cells from melanoblasts or from Langerhans cells of epidermis seems unlikely. Unless one is to believe in the ubiquitous presence of Langerhans cells in numbers undetectable in normal trachea or bladder, one is forced to conclude, as others have (Prunieras, 1969; Wolff and Schreiner, 1970b; Hashimoto and Tarnowski, 1968) that it arises from tissue of mesenchymal origin. Its presence in the lamina propria might also be interpreted as supporting this concept.

No convincing explanation is apparent for the association between stratified squamous epithelium and the presence of Langerhans cells. The present study shows, however, that the conversion of the epithelium of trachea or bladder to keratin-producing stratified squamous cells leads to the appearance of Langerhans cells. Obviously, until the function of these cells becomes understood, this question cannot be resolved.

7. Chick Embryo Skin Differentiation in Chemically Defined Medium.

There have been many reports on the development of chick embryonic

skin in culture (Fell and Mellanby, 1953; Miszurski, 1937; McLoughlin, 1961a,b). However, most of them were done in natural medium, i.e., with plasma clots or medium with addition of serum proteins. Wessells (1961) employed chemically defined medium and reported successful culture and developments of chick embryoskin. Jensen and Mottet (1970) recently reported the observation with the E.M. of the development of chick embryo skin on plasma clot. However, a similar study with the E.M. on tissue cultivated in chemically defined medium is not available.

The results shown here have indicated that chick embryo skin can grow and differentiate in a chemically defined medium (Waymouth MB 752/1) as well as in a natural medium. The changes presented here are comparable to the development of skin in ovo (Parakkal and Matoltsy, 1968; Mottet and Jensen, 1968), or culture in plasma clot (Jensen and Mottet, 1970). The skin cultured in vitro matured much earlier than in ovo. After one day in vitro the morphology of the skin is similar in the stage of development to that of 10-12day embryo in ovo. The development of the intermediate cell layer and a small amount of keratin fibrils characterized this stage (Wessells, 1961; Mottet and Jensen, 1968). After two or four days in culture, the skins are comparable to those of 12-14 or 15-16 days in ovo, respectively. The periderm (including subperiderm)of the skin was highly developed and contained a large number of peridermal granules after six days incubation. The granular layer of the epidermis appeared. The features resembled the skin of a 17-day embryo. Complete cornification, a stage which is attained in ovo at 18-21 days was observed after 8 days in culture.

Although the periderm and epidermis both originate from the single-layered epithelium of the embryo (Hanson, 1947; Parakkal and Matoltsy, 1968) each of these maintains its own population of cells. The periderm disappears one or two days before hatching. In an *in vitro* study, Fitton-Jackson and Fell (1963) claimed that the periderm is usually sloughed off within 48 hours of cultivation. A discrepancy was found between their results and the present investigation. The periderm in this case not only persisted, but showed excellent differentiation. The periderm after six days in culture was highly differentiated and very much thicker than that of the *in ovo* condition. It degenerated only after the epidermis had become completely cornified, a condition which is similar to the *in ovo* study (Wessels, 1961; Mottet and Jensen, 1968).

Wessells (1961, 1962) on the basis of *in vitro* and *in ovo* experiments reported the observation of granules of KH nature in the periderm. Kingsburg, Allen and Rotheram (1953) have compared the staining properties of these granules with trichohyalin granules of the inner root sheath of hair. Peridermal granules were first studied by electron microscopy by Fell (1964). She observed the osmiophilic granules with a mesh-like ultrastructure in periderm of the beak region of the chick. This was later confirmed by Parakkal and Matoltsy (1968) and Mottet and Jensen (1968). Similar structures have been observed in the present investigation. Their origin and mode of formation remains obscure, but they differ morphologically from both KH and trichohyalin granules and can be identified easily by E.M. However, the morphological difference may not necessarily reflect a difference in chemical composition. In this respect, the nature of these granules is perhaps not

incompatible with the histochemical results obtained by other investigators (Wessells, 1961; Kingsburg et al., 1953).

In a recent study Jensen and Mottet (1970), culturing on plasma clot the five-day chick embryo skin for up to 14 days, observed only partial or incomplete cornification. No KH granules or horny cells were observed. A similar conclusion was reached by Fitton-Jackson and Fell (1963) in the study of 13-day chick skin differentiation in vitro. They (Jensen and Mottet, 1970; Fitton-Jackson and Fell, 1963) also stated that the development of the skin is best seen in the epidermal cysts. In the present investigation, no special relationship with the cysts was observed. Normal keratinization and cornification were observed to be independent of cyst formation. The reason of the discrepancy is not known. However, the difference might be attributed to the retaining of peridermal cells in this investigation, that presumably formed a protective layer for the "epidermis proper".

When the cornified chick epidermis is compared with the mammalian skin, a few differences have been observed. The KH granules in the chick epidermis were relatively small and scattered more often in the peripheral cytoplasm. It is probably due to their small size that they are not visible by light microscopy (Andrew, 1958). They have also been overlooked by some investigators using histochemistry and E.M. (Cane and Spearman, 1967; Spearman, 1964, 1966; Fitton-Jackson and Fell, 1963). Recent investigations of the chick embryo (Mottet and Jensen, 1968; Parakkal and Matoltsy, 1968) and hatching chick skin (Matoltsy, 1969) have shown that KH granules are present as in the

the mammalian skin, but they have a size difference. This organelle has consistently been observed during the present investigation. As in the mammalian skin, they are found not only in the cytoplasm but also in the nucleus.

Another topic is the means by which the keratin filaments come to produce fully cornified, or horny cells. The results shown in this investigation indicate that in the chick skin the formation of horny cells is characterized by peripheral condensation of fibrous materials, keratohyalin granules, ribosomes and other organelles, leaving an axial electron light region and lipid droplets. The formation of the horny cell is finalized by the reduction in size of the electron light axial region. A similar result was obtained by Parakkal and Matoltsy (1968) and Matoltsy (1969). In the mammalian skin or in squamous metaplastic tracheal or urinary bladder epithelium, reported in this study, the peripheral condensation of electron dense materials was not observed. The horny cells were filled with a complex of fibrous and amorphous matrix.

MCG are a prominent feature of mammalian skin, and have been observed also in squamous metaplastic tissues (Hicks, 1968; Ghidoni and Campbell, 1969). However, similar structures were not observed in the cultured chick embryo skin. Matoltsy (1969) reported a multigranular body, which he believes to be equivalent to MCG, in the mammalian skin. These multigranular bodies are formed at a time comparable to the time of appearance of the MCG in mammalian skin, and the granules contained in the multigranular bodies are structurally similar to the MCG (Farbman, 1964; Matoltsy, 1966; Odland and Reed, 1967; Wilgram and Weinstock, 1966). This organelle has not been observed in this study,

probably because of the difference in fixatives used, or perhaps because of some effect exerted in culture conditions.

An attempt to induce mucous metaplasia of the chick skin in vitro in the presence of excess vitamin A was not successful. Although keratinization of the epidermis was inhibited, mucous metaplasia was not observed, even when the concentration of vitamin A was increased to 40 i.u./ml. The reasons for this is not known. It is not likely to be due to a deficiency in the medium, which provides excellent development and differentiation of the control skin cultures. All the reported instances of the induction of mucous metaplasia were done with skin cultured on plasma clots (Fell and Mellanby, 1953; Fell, 1957; Fitton-Jackson and Fell, 1963). It is known that vitamin A (retinol) is not stable in chemically defined medium (Fell and Mellanby, 1953). The failure of mucous metaplasia might be due to the early degradation of the vitamin. If this is true, then the addition of serum protein to the medium should, theoretically, have stabilized the vitamin A and consequently mucous metaplasia would have resulted. However, when either McCoys 5a (with 30 percent calf serum) or Waymouth MB 752/1 (with 10 percent calf serum added) were used, similar results were observed. Thus the question of degradation of vitamin A remains unsettled.

Fell (1957) has shown that different results could be obtained with the same tissue when cultured in different vessels (such as Maximow slides or the watch glass method). This point has also been discussed by Hardy (1967). Perhaps one could suppose that the different culture conditions permitted the expression of respones in one set of experiments but not the other.

The last possibility was that, as stated earlier, the persistence of the peridermal cells throughout the period of incubation in the present study, but not in other studies (Fell and Mellanby, 1953; Fell, 1957; Fell and Rinaldini, 1965) may suggest that the shedding of periderm is necessary for the "epidermis proper" to undergo mucous metaplasia when acted upon by vitamin A.

As mentioned in the Review section, Hardy (1968) believes that there might be a critical time (duration in culture) and critical stage in the development of hair follicle when these two conditions are optimal for mucous metaplasia to develop. This hypothesis, when applied to the present result, could mean that the growth rate of chick embryonic skin, if different in plasma clot and in chemically defined medium, could be a critical factor. Perhaps the critical stage of development is reached too early or too late.

8. Mucous Metaplasia of Cheek Pouch Epithelium.

The results of the present studies confirmed the light microscopic observations of Lawrence, Bern and Steadman (1960), Lawrence and Bern (1960), Cavalaris and Krikos (1967) and Cavalaris, Matukas and Krikos (1969), that topical application of vitamin A paraffin pellets to the hamster cheek pouch produces mucous metaplasia.

The metaplastic changes produced by vitamin A pellets appear to be correlated with the duration of treatment. Pronounced mucous metaplasia was observed only after 20-25 days of vitamin A implantation.

The metaplasia induced by vitamin A pellets was of patchy form as reported by the previous investigators (Lawrence and Bern, 1960;

Cavalaris and Krikos, 1967). Mucous cells were present in certain regions but completely absent in the others.

In specimens from animals treated with pellets for 5-10 days, the hyperplastic reaction associated with inflammation was a common occurence. The epithelium devoid of stratum corneum had wide intercellular spaces which appeared to be filled with an exudate. Although the general arrangement of the epithelium remained squamous, the lack of KH granules and the presence of morphologically altered MCG seemed to indicate that the tendency of the epithelium toward keratinization has to some extent been blocked.

Very different features were observed in specimens 15 days after implantation. Instead of being squamous the cells were cuboidal in shape and several layers in thickness. The intercellular spaces were greatly reduced and an inflammatory reaction was not found in most specimens. Keratin fibrils in the cells had largely disappeared. Some small mucous granules made their appearance for the first time in the superficial layers.

Complete mucous metaplasia was observed 20-25 days after pellet implantation at which time typical mucous cells were observed.

The sequence of metaplastic development appears to be (1) hyperplasia and inflammation leading to loss of the stratum corneum, (2) reorganization and (3) production of mucous.

It is interesting that hyperplasia and some inflammatory reaction were still observed in pouches exhibiting mucous metaplasia. This finding is consistent with a dosage gradient of vitamin A within the same pouch. Vitamin A is believed to be released from the paraffin-mineral oil pellet

as small oil globules, and thus certain areas might be exposed to a higher concentration of vitamin A than others.

Gibbs and Cararett (1966) found that in the chinese hamster mitotic activity of the cheek pouch epithelium appears to be localized. They reported clusters of proliferating basal cells. If this result can be applied to golden hamster then the focal nature of mucous metaplasia might be due, in addition to dosage gradient, to differences in susceptibility of the epithelium in different regions.

Cystic or gland-like structures have been observed deep within the connective tissue of the hamster cheek pouch implanted with vitamin A (Lawrence and Bern, 1960; Cavalaris and Krikos, 1967). Since the hamster cheek pouch is characterized by an absence of hair and glands, and is devoid of rete pegs (Gillette, 1957; Handler and Shepro, 1968), the cystic or gland-like structure found by them were probably caused by vitamin A application. However, similar structures have not been observed in the present investigation, perhaps because of the relatively small area of tissue examined under the electron microscope.

Electron microscopic studies of mucous cells produced by hypervitaminosis A in vivo have not been reported. Fitton-Jackson and Fell (1963) reported the effects on chick embryo skin in vitro. However, the quality of their micrographs was not satisfactory. Better preservations of structures were obtained in my specimens of hamster cheek pouch. The findings were comparable to the descriptions of the mucous cells of many mucous glands (Shackleford and Wilborn, 1970a,b; Meyrick and Reid, 1970; Leeson and Leeson, 1968) and of intestine (Freeman, 1962, 1966; Trier and Rubin, 1965). The size and the density of the granules were similar

to those observed in mucous cells of submandibular glands or mucous cells of the intestinal tract. The spongy, fibrillary , appearance of mucous granules has been observed in this report as in that of the submandibular gland. According to Shackleford and Wilborn (1970b) the fibrillary filaments represent mucin molecules. Whether or not this is true requires further clarification. In this conjuction it is interesting to note that modified MCG as seen in Fig. 66 possesses similar fibrillary material, i.e., mucin material. Perhaps one could trace the mucous metaplastic changes as early as to 5-10 days after vitamin A application.

The finding of some dense material in the mucous granules is puzzling. It is likely that the dense material is chemically different from the electron light component. Possibly the difference between these two components reflects an alteration in the activity of the GA related to its different products in keratin producing and mucous producing stages of the cell, in which it produces MCG (and the dense component of the granules) in the keratinizing stage, and mucous granules (the light component) in the other stage. It seems unlikely to the author that the transformation could be completed in one cell generation, but the transformation is a gradual process requiring continuous stimulus and, perhaps, many cell generations.

It is unlikely that the keratin fibrils, after being synthesized, could disappear from the cells. The final mucous cells which contained few keratin fibrils, must be derived from germinal cells, i.e., basal cells. The frequent observation of mitosis in basal cells tends to support this suggestion. How vitamin A acts to divert the differentiation

has no answer at the present time.

The nature of the crystalloid bodies reported in this case is not clear. They have not been reported in any other mucous cells. Their relatively late appearance in this experiment seems to indicate that they might have some relation to the hydrocarbon medium of the implanted pellet. The paraffin used was of low melting point, and mixed with mineral oil. Moreover, some of the crystalloids seemed to be associated with, or enclosed by, globules which could have been hydrocarbon.

9. Mucous Metaplasia of Mouse Vaginal Epithelium.

This is an excellent example of how stimuli act on cells to produce predictable metaplastic changes.

The results indicate that estrogen being a potent hormone that, when injected into castrated mice, effectively stimulates vaginal epithelial proliferation and produces complete cornification only three days after the start of treatment. This is in accord with the results of Cooper et al. (1967) and Barker and Walker (1966). The local application of estrogen has also been reported to produce similar results (Pollard et al., 1966).

When progesterone was administered no proliferation resulted. However, the superficial cells were dramatically affected. After two days of progesterone treatment the superficial cells assumed a columnar shape and showed some evidence of mucous production. Typical mucous cells were produced after four days of progesterone treatment.

The mucous cells of vaginal epithelium produced by progesterone administration were very similar to those produced by hypervitaminosis A

in the cheek pouch . The size and density of their granules were comparable, except that the average number of mucous granules in the vagina seemed greater than in the cheek pouch. The mucous granules often filled the basal region of the cell and isolated the nuclei from the basal cytoplasm. The origin of the mucous granules from the GA was readily demonstrated after either two or four days of progesterone treatment.

The responses differed from that in the cheek pouch in being uniform throughout the superficial cells of the vaginal epithelium. Crystalloid bodies were not found following progesterone treatment.

The mucous cells of the vagina of progesterone-treated animals showed more fibrils than those of the cheek pouch. This was also true of the basal cells. The reason for this difference is not known. Progesterone and estrogen, administered simultaneously, appeared to produce a result in which the effects of each hormone can be identified. The fact that the epithelium greatly increased in thickness through the development of many layers of squamous cells, and that these cells, especially in the deep part of the epithelium contained many keratin fibrils, is consistent with the action of estrogen. On the other hand, in contrast to the effect of estrogen alone, the superficial cells never became cornified. This fact, together with the fact that many granules, although not typical mucous granules, is consistent with the action of progesterone. Thus, progesterone is capable of modifying the action of estrogen, and estrogen is capable of modifying the action of progesterone.

10 Cartilage and Bony Metaplasia

The results of the present study confirm that tenotomy in the rat is an efficient way of producing cartilaginous metaplasia. Pre-cartilaginous differentiation appeared in the regenerating tendon only 10 days after tenotomy and reached its peak at the second or third week, decreasing thereafter. Metaplastic cartilage first appeared at the second week and reached its peak at the fourth week after tenotomy. The decrease in the number of pre-cartilaginous sites corresponded to the increase in the number of the sites of new cartilage. This would indicate that the pre-cartilaginous sites, having been established, were gradually transformed to cartilage. It also indicates that most of the metaplastic sites were initiated in the second or third week after tenotomy. The cells at this stage are presumably more susceptible to the metaplastic stimulus. Some sites of metaplastic cartilage became calcified in the third week. Bony metaplasia was observed only at the end of this period of the experiments.

One week after transplantation the tendons usually underwent degenerative changes and the size of the tendons was reduced by half in the second week.

There were no recognizable metaplastic sites at the central region of the tendon transplanted to a subcutaneous site. However, a few metaplastic sites were still present in the old tendon stumps and in the newly regenerated tendon adjacent to them. Such metaplastic sites appeared not to undergo degeneration; they existed throughout the period of study (four weeks). This would seem to indicate that if the

metaplastic tissues were not affected by degenerative process of the transplantation procedures, they survived and differentiated normally. the existence of calcified cartilaginous and bony metaplastic sites in two-week regenerating tendon grafts is evidence which supports this view. The transformation of cartilage to calcified cartilage and the development of metaplastic bone had presumably occurred after transplantation.

The conclusion was that those metaplastic sites that were not affected by the degenerative changes during early transplantation had survived and maintained their differentiated state within the period of study. That is to say, these metaplastic sites were stable within the period of present experiments.

However, with the exception of the two-week and three-week regenerating tendon grafts, new metaplastic sites usually did not appear after transplantation.

In the case of bone metaplasia induced by urinary bladder mucosal implantation, it has been demonstrated that the existence of the bone is dependent on a viable explant (Mestel and Spain, 1967). The bone was resorbed when the explant was rejected. The bone induced by urinary bladder grafts and tenotomy is different in the sense that the former requires the continuous presence of the stimulus, while the later is stable after it is formed.

(a) Origin of metaplastic cartilage cells.

The main cell type in the regenerating tendon is the fibroblast. As shown in this study, the structure of fibroblasts and pre-chondroblasts is quite similar, with the exception of cell-shape. Fibroblasts could

always be seen adjacent to or surrounding the pre-chondroblasts or chondrocytes. This evidence leaves little doubt that the metaplastic cartilage originated from fibroblasts of the regenerating tendon. This conclusion is in agreement with the results of Anderson and his associates (Anderson and Coulter, 1965, 1967; Anderson, 1967) on chondrogenesis induced by cultured human amniotic cells. Anderson (1967) found that FL amniotic cells were first invested by numerous fibroblasts that were later transformed to cartilage cells. In a recent light microscopic study on cartilage and bone induction by FL cells and another line of human amniotic cells known as WISH, Wlodarski, Hinek and Ostrowski (1970) concluded that the cartilage is derived from the fibroblast-like cells. Other authors studying cartilage and bone induction by urinary bladder implants (Huggins, 1931a; Anspach, 1964; Makin, 1962; Mestel and Spain, 1967) and regenerating tendon (Buck, 1953) held a similar opinion.

Makin (1962) and Trueta (1963) believed that the origin of the metaplastic cells may be related to the endothelial cells of the blood vessels. However, in this case, no spacial relationship between blood vessels and metaplastic sites was observed. It would seem rather unlikely for this to happen in the tendon, although the possibility could not be precluded.

Friedenstein and his associates (Friedenstein et al., 1967; Friedenstein, 1968) designed a system to study bone induction by urinary bladder mucosa within the millipore filter chamber implanted intraperitoneally. When only cells of the bladder mucosa (epithelium and lamina propria) were contained in the chamber, no bone developed, but when leukocytes of peripheral blood, peritoneal fluid cells and spleen

cells were each cultured together with transitional epithelium of bladder, bone was regularly formed within the chamber (Friedenstein et al., 1967). This clearly indicates that tissues of haemopoietic origin contain cells that have the potential for osteogenesis. However, whether or not this could be applied to chondrogenesis and osteogenesis in the regenerating tendon is not clear. From the present ultrastructural study there was no evidence of the direct involvment of these cells in the development of metaplastic cartilage and bone.

(b) Development of metaplastic cartilage.

It is clear from the results that the first step taken by the fibroblast toward a cartilage metaplasia was to retract the cytoplasmic processes and to replace these with serrated or scalloped shaped projections, characteristic of chondroblasts. The nucleus became eccentrically located. This cell might begin to produce cartilage matrix and become a typical chondroblast. These cells might divide and form cell nests or might remain isolated. Calcification of cartilage was observed in specimens obtained three or four weeks after tenotomy. Bone only appeared later (four or five weeks after tenotomy). In short, the stages observed here were from fibroblast to pre-chondroblast, to chondrocyte to calcified cartilage.

(c) Factors that control the metaplasia.

The factors that are involved in the control of cartilaginous and bony metaplasia have been extensively studied by many investigators under the various circumstances (Huggins, 1930a,b, 1931a,b; Buck, 1953; Scapinelli and Little, 1970; Makin, 1962; Anspach, 1964; Friedenstein, 1968; Friedenstein et al.,1967).

In the case of chondrogenesis and osteogenesis following

implantation of urinary bladder mucosa, it was believed that some stimulants might be produced by the explant that induced the metaplasia. Huggins (1931b) thought that a high alkaline phosphatase activity of the explant might be responsible for this change. But experimental work has proved fruitless (Huggins, et al., 1936). Friedenstein (1959) and Makin (1962) noted a PAS positive material closely associated with the bony metaplasia. The amount and extent of PAS positive material is in direct proportion to the size of the metaplastic bone. They believed that the PAS positive material might be the osteogenic substance. When the urinary bladder mucosa was isolated from the fascia with a millipore filter, bony metaplasia was not observed. This led Makin (1962) to believe that the actual contact of the transitional epithelium with the facia is a factor in this form of induction.

Friedenstein et al. (1967) and Friedenstein (1968), on the other hand, have reported success in induction of bony metaplasia in the fascia by urinary bladder mucosa when the later was isolated by millipore chambers. This would tend to indicate that it is some substance produced by the transitional epithelium that is responsible for the induction and not the direct physical contact as suggested by Makin (1962). The nature of the substance is unknown.

In the case of tendon a similar osteogenic substance is not likely to be present. Tensile forces have been suggested as initiators of the metaplasia (Buck, 1953; Scapinelli and Little, 1970) in tendons and ligaments. There was much lower frequency of cartilaginous metaplasia in the regenerating tendon when the leg was immobilized (Buck, 1953). In the present study the fact that the tendon grafted

at one week of regeneration failed to develop cartilage tends to indicate that tensile forces are important in the development of metaplasia. This idea is in agreement with the view of many earlier investigators (Thomas, 1913; Carey et al., 1927-28; Krompecher, 1937).

In normal human subjects cartilage metaplasia has been observed in vertebral interspinous ligaments (Scapinelli, 1960a,b, 1963) and in the tendons of other regions (Scapinelli and Little, 1970). However, the changes in these cases were much slower than in the regenerating tendon, taking years to be completed. Scapinelli and Little (1970) believe that in the case of normal tendon and ligament, a combined compression and rotation are responsible for the metaplastic changes.

The reason for tensile forces inducing cartilaginous and bony metaplasia is not known. It may be that the regenerating tendon has a need of stronger reinforcement to sustain the strong tensile pull constantly exerted from the muscle. The fact that relatively few new metaplastic sites were elicited after the third or fourth week, by which time the strength of the regenerating tendon is great, may be cited as support for this idea.

(d) Cytofilaments.

The structure of the chondrocytes of the present study was strikingly at variance with previous reports (Godman and Porter, 1960; Godman and Lane, 1964; Revel and Hay, 1963; Fitton-Jackson, 1964) in respect of the large number of cytofilaments. Although cytofilaments have been observed in chondrocytes (Göthlin and Ericsson, 1970; Anderson, Chacko, Abbott and Holtzer, 1970) and many other fibrogenic and non-

fibrogenic cells (Buckley and Porter, 1967), their prominence did not approach that reported here. Cytofilament (as distinct from filaments of cortical cytoplasm) observed in this case were mainly perinuclearly located and usually appeared in large bundles. They occurred with a lower frequency in other parts of the cytoplasm as isolated filaments, or in small bundles. Morphologically, they were similar to those observed in other chondrocytes and in other cell types (Buckley and Porter, 1967). They were about 100A° in diameter and had no banding. The reason for the presence of the enormous number of cytofilaments in the metaplastic cells is not known. In other cell types the cytofilaments are believed to have an effect on cytoplasmic viscosity, or to act as a cyto-skeleton (Buckley and Porter, 1967; Anderson et al., 1970). In still other cases, cytofilaments appear to be actively involved in movements seen in rat embryo cells (Buckley and Porter, 1967) or in chondrocytes treated with 5 —bromodeoxyuridine (Anderson et al., 1970). Cytoplasmic filaments are found in amoeba (Nachinias, 1964), in cultured cells (Golberg and Green, 1964; Taylor, 1966), and in various types of blood cells known to exhibit amoeboid movement (Bessis and Breton-Gorries, 1965).

It seems unlikely that the cytofilaments observed in chondrocytes have anything to do with cell motility. The presence of filaments is more likely to be supportive.

Another interesting feature is the observation of an increase in thickness of the cortical fibrillary zone as the cells differentiated from fibroblast to chondrocytes. The filaments observed in this region were generally smaller than the cytofilaments discussed in the previous paragraph, averaging 50-80A° in diameter. They have been commonly found

in fibrogenic cells, including chondrocytes (Kagikawa et al., 1959; Yardley et al., 1960; Ross and Benditt, 1961; Ross, 1968; Göthlin and Ericsson, 1970). When the section was cut tangentially to the membrane of the cell the limiting membrane became unrecognizable and the fibrillary zone appeared to be continuous with the matrix outside the cell. On the basis of this type of morphologic evidence (Yardley et al., 1960; Yardley, 1962; Godman and Porter, 1960; Chapman, 1961) it has been suggested that the collagenous fibrils are assembled in the ectoplasm of the fibroblast and shed by disintegrating the overlying cell membrane. But this concept of fibrillogenesis has not been supported by most of the later workers (Goldberg and Green, 1964; Revel and Hay, 1963; Salpeter, 1968; Göthlin and Ericsson, 1970). The current theory of fibrillogenesis is that the precursor (tropocollagen) is secreted by the cell and assembled extra-cellularly. Therefore, the function of fibrillary zone observed in fibroblasts and chondrocytes in this investigation, and previously reported, is probably not a collagen precursor. It is more likely that these filaments have supporting function.

The observation of fibrillary material and sometimes collagen-like fibres in the cytoplasmic vesicles of the fibroblasts and chondrocytes is interesting. It is believed as others (Revel and Hay, 1963; Sheldon, 1964; Ross, 1968) have suggested that the fibrillary material in the vesicles is the precursor of the collagen, or matrix fibrils of cartilage. Mature collagen fibres have seldom been observed in the vesicles. Welsh (1966) found collagen-like fibres within neoplastic fibroblasts. Göthlin and Ericsson (1970) reported the observation of

collagen-like fibres within various cell types (including fibroblasts, chondroblasts and osteoblasts) in the fracture callus. They believed that the occurrence of collagen-like fibres within the cell represents a minor deviation in the maturation process of collagen. The observation of a similar phenomenon in the present investigation tends to support the view of Göthlin and Ericsson (1970). The reason might be that the demand of active synthesis of collagen fibres was so great that some of them were assembled while still in the cell.

(e) Matrix of metaplastic cartilage

The matrix of metaplastic cartilage, as observed in this investigation, differs from hyaline cartilage matrix in several respects. Firstly, the matrix of the metaplastic cartilage is much more scanty. Typical cartilage matrix, which consists of matrix fibrils, amorphous material and dense matrix granules (Godman and Porter, 1960; Revel and Hay, 1963; Takuma, 1960; Matukas et al., 1966; Khan and Overton, 1970) was observed only in a relatively narrow zone surrounding the chondrocytes. Further away, the matrix was composed mainly of collagenous fibrils, intermingled with some matrix materials. Typical matrix was observed uniformly between chondrocytes only when they were in the same nest. Secondly, the collagen fibrils in the metaplastic cartilage were much more abundant and although finer than in non-metaplastic regions of regenerating tendon, they were larger than in hyaline cartilage. The fibrils were in most cases, regularly distributed in contrast to the random distribution in hyaline cartilage. Finally, the dense matrix granules which are another prominent feature of hyaline cartilage, were found in much smaller number in the metaplastic tissue. Similar

matrix granules have also been observed by Anderson (1967) in the metaplastic cartilage induced by cultured human amniotic cells. It has been suggested that the granules represent mucoprotein (Revel and Hay, 1963) or mineral deposits (Godman and Porter, 1960). More recently, it has been shown that the granules represent a protein-polysaccharide complex, including chondroitin sulphate (Matukas et al., 1966; Khan and Overton, 1970).

The matrix fibrils in the metaplastic cartilage were small, randomly orientated, and without cross-banding. Aggregates of fibrils also without cross-banding, were occasionally observed with a specific orientation in respect to the cell. They lay parallel to the plasma membrane to form a basement membrane-like structure.

(f) Matrix vesicles.

Matrix vesicles with a morphology and distribution similar to those in metaplastic cartilage have been reported in the epiphyseal cartilage (Anderson, 1969; Thyberg and Friberg, 1970). Since in earlier reports on cartilage no mention of such vesicles was found, it remains to be determined whether this is a common feature for all types of cartilage. The matrix vesicles, as observed in the present investigation, and also by Anderson (1969) were usually distributed between chondrocytes, but at some distance away from them. They were seldom found in the lacunae.

The observation of cell organelles in the vesicles leaves little doubt that these vesicles have their origin intracellularly. It is probably correct to say that they originate by budding off from the cytoplasmic processes. In a few instances, bands of vesicles could be seen extending out from the chondrocytes, lending support to the above explanation. Another possibility is that the matrix vesicles were

dispersed from disintegrating chondrocytes. This appeared unlikely because dying or disintegrating chondrocytes have never been observed in non-calcifying cartilage. It would appear that the "cytoplasmic fragments" reported by Anderson (1967) in induced (metaplastic) cartilage may be comparable to the matrix vesicles observed here.

The function of these matrix vesicles is not known. Anderson (1969) reported a close relationship between apatite crystallites and the vesicles in the calcifying epiphyseal cartilage, and suggested that the matrix vesicles might play a role in the initiation of calcification. However, such a relationship has not been observed in metaplastic cartilage, either in the present study or in Anderson's (1967) study.

(g) Calcification

Calcification of the metaplastic cartilage of the regenerating tendon appeared to be similar to that reported for the epiphyseal cartilage (Takuma, 1960; Robinson and Cameron, 1956; Anderson, 1964) and for induced cartilage (Anderson, 1967). The apatite crystallites first appeared in small groups in the matrix of the cartilage and later grew in size, eventually to form a confluent mass. Concurrent with this process was the hypertrophy and degeneration of the cartilage cells.

The apatite crystallites were, in some cases, closely associated with collagen fibrils, but in others they developed independently from them. They developed in the matrix between the chondrocytes. Collagen fibrils are generally considered as structures important for crystal nucleation in calcification (Cameron, 1963). However, Bonucci (1967, 1969) and Decker (1967) reported no essential relationship between nucleation and collagen fibrils, and this is in accord with the findings

of the present investigation. Initial calcium deposition could occur in matrix independently of collagen. Sherft (1968) found no collagenous fibrils in decalcified regions of matrix and concluded that collagenous fibrils have no significance in nucleation.

Another type of nucleus for apatite growth, consisting of a needle-like organic core (apatite crystallite ghost) similar in shape and size to the apatite crystals, has been described by Bonucci (1967, 1969). They could be visualized in the decalcified tissue after staining with uranylacetate and lead citrate, but proved negative with the electron diffration method. In addition, they were removed by papain digestion. Appleton (1970) using chromium as demineralizer, was able to demonstrate the crystallite ghosts without other staining. He believed that chromium was able to substitute calcium in the crystallites. However, the chemical nature of the crystallite ghosts and their role in calcification requires further investigation.

It has recently been reported that mitochondria play a role in concentrating calcium salts in calcification (Martin and Matthews, 1969). These workers demonstrated that in the epiphyseal cartilage the number of mitochondrial granules is in direct proportion to the maturation of the chondrocytes, being few in proliferative cells (1.5 per M), increasing in maturation zones (6.3 per M) and hypertrophic zones.

Electron microscopic autoradiographic study using C^{47} indicates a rapid cellular and mitochondrial incorporation of calcium. The loss of mitochondrial granules corresponds with the onset of calcification (Matthews et al., 1968; Martin and Matthews, 1969; Matthews, 1970). Shapiro and Greenspan (1969) have suggested that mineralization may be

under strict cellular control, and that it is the mitochondria which are responsible for concentrating the principal inorganic ions to the crystalline phase. They also suggested that during mineralization these ions are transported from the mitochondria to the extracellular matrix.

Although mitochondrial granules have been observed in the chondrocytes in the present study, their number was small. The role of mitochondria in calcification under these circumstances is not known.

11. Cilia

Cilia, once attributed solely to epithelial tissues (Barnes, 1961; Fawcett and Porter, 1954) have now been observed in many other types of cells. Scherft and Daems (1967) reported the observation of single cilia in chondrocytes of epiphyseal cartilage. Solitary cilia have also been reported in fibroblasts (Sorokin, 1962; Schuster, 1964) or fibroblasts treated with colcemid (Stubblefield and Brinkley, 1966) and in smooth muscle cells (Sorokin, 1962). Recently, single cilia have been reported in cardiac muscle (Manasek, 1968) and in embryonic heart muscle (Rash et al., 1969). In the nervous system Taxi (1961) observed cilia in neurons of the sympathetic nervous system and Grillo and Palay (1963) in the Schwann cells. Palay (1961) and Del Cerro and Snider (1967) demonstrated cilia in neurons of the central nervous system. Milhaud and Pappas (1968) reported the observation of cilia in neural and neuroglial cells in cat brain after pargyline treatment. Single cilia have also been reported in endocrine cells (Boquist, 1970). Obviously, the potential for cilia formation is

present in a great variety of cell types, and is expressed by environmental changes. The stimuli for metaplasia are, apparently, examples of this type of environmental change.

The significance of the development of these cilia is not known. Rash et al. (1969) correlated the possession of cilia and the interphase stage of the mitotic cycle. They believed that the abrupt transformation from mitotic replicative tissue to non-mitotic structuring tissues is correlated with the disappearance of centrioles and the formation of cilia. In their opinion, mitotic activity and the possession of cilia are mutually exclusive attributes. It appears that mitotic inhibition, which is under nuclear control, is somehow mediated by, or results in, the observed centriole transformation as an alternate pathway for centriole activity. It is probably true that cells with even a single cilium do not divide, but whether or not this is related to the loss of centrioles is a matter of speculation.

The author endorses the idea that single cilia formation is a common reaction of cells toward an adverse environment, as it is commonly observed under the present experimental conditions.

The presence of a ciliated cell among mucous metaplastic cells of the cheek pouch epithelium was a great surprise, although Fell and Mellanby (1953) observed ciliated cells in skin treated with excess vitamin A in vitro. Aydelotte (1963) observed a similar phenomenon in esophageal cells cultured in hypervitamin A medium. The presence of the ciliated cell in this case may be directly related to the excess of this vitamin.

IV. SUMMARY

(1) The tracheal epithelium of rats maintained on vitamin A deficient diet was studied for the first time by electron microscopy. The first morphological evidence of the development of metaplasia was the appearance of clusters of hyperplastic basal cells, several such foci being present in one animal. The areas of hyperplasia then extended; the respiratory epithelium superficial to these cells was undercut, and its cells were desquamated.

The loosely associated hyperplastic cells differentiated into flatter cells towards the surface and showed the specific features of cornifying epithelium, such as membrane coating granules (MCG), keratohyalin granules and keratin fibrils. Finally, there was a shrinking of the intercellular spaces and the further development of desmosomes as well as the appearance of layers of fully differentiated horny cells on the surface.

(2) It is concluded that, under the conditions of these experiments, the metaplastic transformation of the epithelium does not involve a de-differentiation of mature cells, but is the result of the differentiation of generative cells in a new direction.

(3) Squamous metaplasia in urinary bladder epithelium of vitamin A deficient rats was also preceded by hyperplasia. It differed from hyperplastic tracheal epithelium in that, hyperplastic basal cell nests were not observed. Regions of semi-cornified epithelium were found, in which the relatively normal hyperplastic bladder epithelium was seen lying atop the keratinizing epithelium. The surface non-cornifying epithelium showed several morphological variations, such as a decrease in the number of fusiform vesicles or the production of modified (type I and type II) vesicles. The modified vesicles resembled fusiform vesicles in respect of asymmetric unit membrane but resembled the membrane-coating granule in shape, size and location. The continuum of morphologic variations of the vesicles reflected the functional changes of the superficial cells of the semi-cornified bladder epithelium. The golgi apparatus of the superficial cells, while returning the capability for synthesizing asymmetric unit membrane, also produced membrane-coating granule-like matrix. Keratohyalin granules were also found in these cells.

(4) It is believed that the initial superficial cells were not eventually cornified, and hence, were not part of the final cornified epithelium. The cells of the stratum corneum are formed by differentiation of the keratinizing base.

(5) The cornified bladder epithelium showed closer resemblance to the epidermis than the metaplastic tracheal epithelium. In

addition to all the characteristic features of epidermis, two morphologically distinct types of keratohyalin granules were reported.

(6) The peculiar asymmetrical plasma membrane of the normal urinary bladder was heavily stained by ruthenium red, as were those fusiform vesicles which had continuity with the plasma membrane.

(7) Langerhans cells, normally found in epidermis, were observed for the first time in epidermoid epithelium and lamina propria of the trachea and urinary bladder of vitamin A deficient rats. This could be considered as single cell metaplasia.

(8) Chick embryo skin cultured in chemically defined medium was studied for the first time by electron microscopy. Excellent development and differentiation of chick embryo skin was recorded. Keratohyalin granules, which were claimed to be absent in avian skin, were observed.

An alternative pattern of keratinization, which is characterized by cortical condensation of the fibrillary material and keratohyalin granules, confirmed the recent observation of Matoltsy (1969) made on the same system in ovo.

Complete mucous metaplasia of chick embryo skin cultured in medium with excess vitamin A was not observed, but the keratinization of the epidermis was inhibited.

(9) Mucous metaplasia of hamster cheek pouch epithelium induced by vitamin A pellet implantation was studied for the first time by electron microscopy. After 5-10 days of vitamin A treatment, the epithelium was devoid of stratum corneum, and usually infiltrated with some inflammatory cells. The intercellular spaces were wide, and membrane-coating granules underwent modifications believed to be the earliest signs of mucous production. After 15 days of vitamin A treatment the inflammation had largely gone, and the epithelium was made up of several layers of cuboidal cells. The intercellular spaces and keratin fibrils had greatly decreased. Some mucous granules appeared for the first time in the cuboidal superficial cells. Various other changes in the cheek pouch epithelium were observed in animals treated with vitamin A for 20-25 days. In some regions hyperplasia or inflammatory reactions were observed, whereas in other regions complete mucous metaplasia was found. In completely metaplastic regions a surface layer of typical mucous cells was seen lying atop two or three layers of cuboidal cells.

(10) A peculiar type of bar-shaped crystalloid bodies, observed first in 15-day specimens of cheek pouch epithelial cells, was found in mucous cells, macrophages, and rarely, in basal cells. They probably originated from the implanted paraffin-mineral oil suspending vehicle.

(11) Although the vaginal epithelium of castrated mice was made up of 3-4 layers of cuboidal cells, when the castrated mice were treated with estrogen, the vaginal epithelium proliferated and quickly cornified.

No proliferation was elicited when mice were treated with progesterone. The superficial cells became columnar and began mucous production.

Typical mucous cells were observed uniformly over the surface after four days of progesterone injection.

(12) When estrogen and progesterone were injected simultaneously into castrated mice, the action of both hormones was reflected in the epithelium. The epithelium greatly increased in thickness, synthesizing keratin fibrils on the one hand, and remaining non-cornified, and producing mucous (although morphologically modified) granules on the other. Columnar cells, with small numbers of mucous granules, were sometimes found among the surface squamous non-cornifying vaginal epithelial cells.

(13) A quantitative study of cartilaginous metaplasia was done on the regenerating Achilles tendon. The pre-cartilaginous sites were observed in ten-day regenerating tendon, and their number reached a peak in the second or third week.

A few sites of metaplastic cartilage were found in the second week, increased dramatically in the third week, and reached a peak at the fourth week, by which time the number of pre-cartilaginous

sites had decreased.

Calcification of sites of cartilaginous and bony metaplasia was observed at the third and fourth weeks respectively.

(14) Transplantation of regenerating tendon indicated that the metaplastic change to cartilage of the regenerating tendon was stable within the period of study.

(15) Electron microscopic study of the metaplastic cartilage in the rat regenerating tendon was recorded for the first time. The morphological transition from fibroblast, through precondroblast, to chondrocyte was clearly demonstrated. The metaplastic cartilage differed in various respects from the normal cartilage such as in the presence of enormous numbers of cytofilaments, in thin scanty matrix and smaller number of matrix granules, and in their larger and more abundant collagen fibrils.

(16) Early calcification in regenerating tendon was observed as radiating clusters of electron dense, needle-like crystals deposited randomly in the matrix. These calcification sites later spread, and the crystallites formed confluent masses.

(17) Single cilia have been observed in all the tissues studied in the present investigation, including a cilium in a metaplastic chondrocyte and a ciliated cell from cheek pouch epithelium treated with vitamin A.

APPENDIX

Waymouth's Medium MB 752/1

Ingredients	Concentration (mg/100ml)	Ingredients	Concentration (mg/100ml)
Glucose	500.0	L-Lysine-HCl	24.0
Ascorbic acid	1.75	L-Histidine-HCl	15.0
Cysteine-HCl	9.0	L-Glutamic acid	15.0
Glutathione	1.5	L-Threonine	7.5
Choline-HCl	25.0	L-Arginine-HCl	7.5
Hypoxanthine	2.5	L-Valine	6.5
Glutamine	35.0	L-Aspartic acid	6.0
Thiamine-HCl	1.0	Glycine	5.0
Calcium pantothenate	0.1	L-Proline	5.0
Riboflavine	0.1	L-Leucine	5.0
Pyridoxine-HCl	0.1	L-Methionine	5.0
Folic acid	0.04	L-Tyrosine	4.0
Biotin	0.002	L-Phenylalanine	5.0
m-Inositol-$2H_2O$	0.1	L-Tryptophan	4.0
Nicotinamide	0.1	L-Isoleucine	2.5
Vitamin B_{12}	0.02	L-Cystine	1.5

REFERENCES

Abehouse, B.S. & Tankin, L.H. (1956). Leukoplakia of the renal pelvis and bladder. J. Urol. 76: 330-337.

Ackerman, L.V. (1946). Mucinous adenocarcinoma of the pelvis of the kidney. J. Urol. 55: 36-45.

Ackerman, N.B. (1968). Bone formation in the gastric mucosa following subtotal gasterectomy in rats. Exp. Molec. Path. 9: 125-130.

Albright, J.T. & Listgarten, M.A. (1962). Observations of the fine structure of the hamster cheek pouch epithelium. Arch. Oral Biol. 7: 613-620.

Alvarez-Ierena, J. De J. (1952). Physiology of the transitional epithelium of the urinary tract. I. Vesical secretion in the dog. J. Urol. 67: 939-950.

Amprino, R. & Bairati, A. (1934). Cited from: Scapinelli, R. & Little, K. (1970). Observations on the mechanically induced differentiation of cartilage from fibrous connective tissue. J. Path. 101: 85-91.

Anderson, D. R. (1964). The ultrastructure of elastic and hyaline cartilage of the rat. Am. J. Anat. 114: 403-433.

Anderson, H. C. (1967). Electron microscopic studies on induced cartilage development and calcification. J. Cell Biol. 35: 81-101.

Anderson, H.C. (1969). Vesicles associated with calcification in the matrix of epiphyseal cartilage. J. Cell Biol. 41: 59-72.

Anderson, H.C., Chacko, S., Abbott, J. & Holtzer, H. (1970). The loss of phenotypic traits by differentiated cells in vitro. Am. J. Path. 60: 289-311.

Anderson, H.C. & Coulter, P.R. (1965). Induction of cartilage and bone formation in mice by transplanted FL human tissue culture cells. Federation Proc. 24: 437-

Anderson, H.C.& Coulter, P.R. (1967). Bone formation induced in mouse thigh by cultured human cells. J. Cell Biol. 33: 165-177.

Anderson, H.C., Merker, P.C. & Foch, J. (1964). Formation of tumours containing bone after intramuscular injection of tansformed human amnion cell (FL) into cortisone treated mice. Am. J. Path. 44: 507-519.

Andrew, W. (1959). Textbook of Comparative Histology. Oxford University Press, London and New York.

Angrist, A., Capurro, P. & Moumgis, B. (1960). Studies on squamous metaplasia in rat bladder. II. Effects of estradiol and estradiol plus hexestrial. Cancer Res. 20: 568-572.

Anspach, W.E. (1964). Bone formation induced by epithelium of urinary bladder. Arch. Surg. 89: 446-456.

Appleton, J. (1970). Ultrastructural observations on early cartilage calcification. The use of chromium sulphate in decalcification. Calc. Tiss. Res. 5: 270-276.

Armstrong, C.P., Harlin, H.C. & Fort, C.A. (1950). Leukoplakia of the renal pelvis. J. Urol. 63: 208-213.

Arons, P. & van der Rijst, M.P.J. (1932). Cause of infections in cases of vitamin A deficiency. Arch. Nederl de Phys. 17: 578-613.

Asami, G. & Dock, W. (1920). Experimental studies on heteroplastic bone formation. J. Exp. Med. 32: 745-766.

Auerbach, O., Gene, J.B., Forman, J.B., Petrick, T.G., Smolin, H.J. Meuhsam, G.E., Kassouny, D.Y., & Stout, A.P. (1957). Changes in bronchial epithelium in relation to cigarette smoking and cancer of the lung. New Eng. J. Med. 256: 97-104.

Auerbach, O., Hammond, G.C., Kirman, D., Garfinkel, L. & Stout, A.P. (1957). Histologic changes in bronchial tubes of cigarette smoking dogs. Cancer. 20: 2055-2066.

Auerbach, O., Petrick, T.G., Stout, A.P., Statsinger, A.L., Muehsam, G.E., Forman, J.B., & Gene, J.B. (1956). The anatomical approach to study of smoking and bronchogenic carcinoma: Preliminary report of 41 cases. Cancer. 9: 76-78.

Auerbach, O., Stout, A.P., Hammond, E.C., & Garfinkel, L. (1961). Changes in bronchial epithelium in relation to cigarette smoking and in relation to lung cancer. New Eng. J. Med. 265: 253-267.

Auerbach, O., Stout, A.P., Hammond, E.C., & Garfinkel, L. (1962a). Changes in bronchial epithelium in relation to sex, age, residence, smoking and pneumonia. New Eng. J. Med. 267: 111-119.

Auerbach, O., Stout, A.P., Hammond, C. & Garfinkel, L. (1962b). Bronchial epithelium in former smokers. New Eng. J. Med. 267: 120-125.

Aydelotte, M.B. (1963). The effects of vitamin A and citral on epithelial differentiation. 2. The chick esophageal and corneal epithelia and epidermis. J. Embryol. Exp. Morphol 11: 621-635.

Barker, T.E. & Walker, B.E. (1966). Initiation of irreversible differentiation in vaginal epithelium. Anat. Rec. 154: 149-160.

Barnes, B.G. (1961). Ciliated secretory cells in the pars distalis of the mouse hypophysis. J. Ultrastruct. Res. 5: 453-467.

Bartelsen, A. (1944). Experimental investigations into post-foetal osteogenesis. Acta Orthopaed. Scand. 15: 139-181.

Bassett, B. & Packer, L. (1965). Response of isolated lysosomes to vitamin A. J. Cell Biol. 27: 448-450.

Beaver, D.L. (1961). Vitamin A deficiency in the germ-free rat. Am. J. Path. 38: 335-357.

Beitch, I. (1970). The induction of keratinization in the corneal epithelium. Invest. Ophthalmol. 9: 827-843.

Berkheiser, S.W. (1959). Bronchiolar proliferation and metaplasia associated with bronchiectasis pulmonary infarcts and anthracosis. Cancer 12: 438-508.

Besemann, E.F. (1967). Renal leukoplakia. Radiology 88: 872-877.

Bessis, M. & Breton-Gories, J. (1965). Cited from: Buckley, I.K. & Porter, K.R. (1967). Cytoplasmic fibrils in living cultured cells. A light and electron microscope study. Protoplasma 64: 349-380.

Birbeck, M.S.C., Breathnach, A.S. & Everall, J.D. (1961). An electron microscopic study of basal melanocytes and high level clear cells (Langerhans cells) in vitilgo.

J. Invest. Dermatol. 37: 51-67.

Black, H. & Ackerman, L.V. (1952). The importance of epidermoid carcinoma in situ in the histogenesis of carcinoma of the lung. Am. Surgeon. 136: 44-55.

Blessing (1959). Cited from: Makin, M. (1962). Osteogenesis induced by vesical mucosal transplant in the guinea-pig. J. Bone Joint Surg. 44B: 165-193.

Bloom, W. & Fawcett, D.W. (1968). A textbook of histology. 9th edition. W.B. Saunders Co., Philadelphia. Chp. 9 & 10: 212-262.

Bohatirchuk, F. P. (1965). The study of calcification of mammalian cartilage in normal and pathology by stain historadiography. Am. J. Anat. 117: 287-310.

Bohatirchuk, F.P. (1969). Metaplasia of cartilage into bone: A study by stain historadiography. Am. J. Anat. 126: 243-254.

Bonneville, M.A., Weinstock, M. & Wilgram, G.F. (1968). An electron microscope study of cell adhesion in psoriatic epidermis. J. Ultrastruct. Res. 23: 15-43.

Bonucci, E. (1967). Fine structure of early cartilage calcification. J. Ultrastruct. Res. 20: 33-50.

Bonucci, E. (1969). Further investigation of the organic/inorganic relationships in calcifying cartilage. Calc. Tiss. Res. 3: 38-54.

Boquist, L. (1970). Cilia and vesicular particles in the endocrine pancreas of the mongolian gerbil. J. Cell Biol. 45: 532-541.

Breathnach, A.S. (1964). Observations on cytoplasmic organelles in

Langerhans cells of human epidermis. J. Anat. 98: 265-270.

Breathnach, A.S. (1965). The cell of Langerhans. Intern. Rev. Cytol. 18: 1-28.

Breathnach, A.S. & Wyllie, L.M. (1964). Electron microscopic observations on human skin impregnated with gold chloride. J. Anat. 98: 289.

Breathnach, A.S., Silvers, W.M., Smith, J. & Heyner, S.P. (1968). Langerhans cells in mouse skin experimentally deprived of itsneural crest component. J. Invest. Dermatol. 50: 147-160.

Bridges, J.B. & Pritchard, J.J. (1958). Bone and cartilage induction in the rabbits. J. Anat. 92: 28-38.

Brody, I. (1959). An ultrastructural study on the role of the keratohyalin granules in the keratinization process. J. Ultrastruct. Res. 3: 84-104.

Brody, I. (1962). The ultrastructure of the epidermis in psoriasis vulgaris as revealed by electron microscopy. (3) Stratum inter-medium in parakeratosis without keratohyalin granules J. Ultrastruct. Res. 6: 341-353.

Brody, I. (1964). Different staining methods for the electron microscopic elucidation of the tonofibrillar differentiation in normal epidermis. In. Montagna, W. & Lobitz, W.C. ed. The Epidermis. Academic Press, New York. 251-280.

Brody, I. (1970). An electron microscopic study of the fibrillar density in the normal human stratum corneum. J. Ultrastruct. Res. 30: 209-217.

Buck, R.C. (1953). Regeneration of Tendon. J. Path. Bact. 66: 1-18.

Buckley, I. & Porter, K. (1967). Cytoplasmic fibrils in living cultured cells. A light and electron microscopic study. Protoplasma 64: 349-380.

Bunting, C.H. (1906). The formation of true bone with cellular (red) marrow in the sclerotic aorta. J. Exp. Med. 8: 365-376.

Burian, K. (1960). Cited from: Symchych, P.S. & Cadotte, M. (1967). Squamous metaplasia and necrosis of the trachea complicating prolonged nasotracheal intubation of small newborn infants. An autopsy study. J. Pediat. 71: 541-543.

Cameron, D.A. (1963). The fine structure of bone and calcified cartilage. A critical review of the contribution of electron microscopy to understanding of osteogenesis. Clin. Ortho paed. 26: 199-228.

Cane, A.K. & Spearman, R.I.C. (1967). A histochemical study of keratinization in the domestic fowl Gallus gallus. J. Zool. London 153: 337-352.

Capurro, P., Angrist, A., Black, J. & Moumgis, B. (1960). Studies in squamous metaplasia in rat bladder. (II) Effects of hypovitaminosis A, foreign bodies and methylcholanthrene. Cancer Res. 20: 563-567.

Carey, E.J., Zeit, W. & McGrath, B.F. (1927-28). Studies in the dynamics of histogenesis. XII. The regeneration of the patellae of dogs. Am. J. Anat. 40: 127-158.

Cavalaris, C.J. & Krikos, G.A. (1967). Vitamin A produced mucous metaplasia. J. Oral Therap. Pharmacol 3: 452-461.

Cavalaris, C.J., Matukas, V.J. & Krikos, G.A. (1969). Histochemistry of the mucins of vitamin A produced mucous metaplasia in hamsters. Arch. Oral Biol. 14: 1313-1322.

Ceccarelli, G. (1931). Cited from: Makin, M. (1962). Osteogenesis induced by vesical mucosal transplant in the guinea-pig. J. Bone Joint Surg. 44B: 165-193.

Chang, S.C. (1957). Microscopic properties of whole mounts and sections of human bronchial epithelium of smokers and non-smokers. Cancer. 10: 1246-1262.

Chapman, J.A. (1961). Morphological and chemical studies of collagen formation. I. The fine structure of guinea-pig granulomata. J. Biohys. Biochem. Cytol. 9: 639-651.

Christopher, E. & Braun-Falco, O. (1968).Cited from: Olson, J.A. (1969) Metabolism and function of vitamin A. Federation Proc. 28: 1670-1677.

Connery, D.B. (1953). Leukoplakia of the urinary bladder and its association with carcinoma. J. Urol. 69: 121-127.

Constance, T.J. (1954). Localized myositis ossificans. J. Path. Bact. 68: 381-385.

Cooper, R.A., Cardiff, R.D. & Wellings, S.R. (1967). Ultra-structure of vaginal keratinization in estrogen treated immature Balb/cCrgl. mice. Z. Zellforsch. 77: 377-403.

Courrier, R. (1924). Cited from: Meyer, R.K. & Allen, W.W. (1933). The production of mucified cells in the vaginal epithelium of certain rodents by oestrin and by corpus luteum extracts. Anat. Rec. 56: 321-343.

Craig, L.G. (1939). Cystitis cystica glandularis. J. Urol. 42: 1197-1203.

Daniel, M.R., Dingle, J.T., Glauert, A.M. & Lucy, J.A. (1966). The action of excess vitamin A alcohol on the fine structure of rat dermal fibroblasts. J. Cell Biol. 30: 465-475.

Decker, J.D. (1967). An electron microscopic investigation of osteogenesis in the embryonic chick. Am. J. Anat. 118: 591-614.

De Duve, C., Wattiaux, R. & Wibo, M. (1962). Effects of fat-soluble compounds on lysosomes in vitro. Biochem. Pharmacol. 9: 97-116.

Del Cerro, M.P. & Snider, R.S. (1967). Cilia in the cerebellum of immature and adult rats. J. Microscopie 6: 515.

Della Porta, G., Kalb, L. & Shubik, P. (1958). Introduction of trachiobronchial carcinoma in Syrian hamster. Cancer. Res. 18: 592-596.

DeLuca, H.F., Manett, M.R., Madsen, N. & Olson, E.B. (1963). Action of vitamin A on liver homogenate oxidation of tricarboxylic acid cycle intermediates. J. Nutr. 81: 283-286.

Dingle, J.T. (1961). Studies on the mode of action of excess of vitamin A. 3. Release of a bound protease by the action of vitamin A. Biochem. J. 79: 509-512.

Dingle, J.T., Glauert, A.M., Daniel, M. & Lucy, J.A. (1962). Vitamin A and membrane systems. I. The action of the vitamin on the membrane of cells and intracellular particles. Biochem. J. 84: 76p.

Dingle, J.T. & Lucy, J.A. (1962). Studies on the mode of action of excess of vitamin A. 5. The effect of vitamin A on the stability of the erythrocyte. Biochem. J. 84: 611-621.

Dingle, J.T., Lucy, J.A. & Fell, H.B. (1961). Studies on the mode of action of excess of vitamin A. 1. Effect of excess of vitamin A on the metabolism and composition of embryonic chick-limb cartilage grown in organ culture. Biochem. J. 79: 497-500.

Eddy, E.M. & Walker, B.E. (1969). Cytoplasmic fine structure during hormonally controlled differentiation in vaginal epithelium. Anat. Rec. 164: 205-218.

Elfving, G. & Hästbacka, J. (1965). Pancreatic heterotopia and its clinical importance. Acta Chir. Scand. 130: 593-602.

Fairbank, H.A.T. (1950). Myositis ossificans progressiva. Synomyms-Fibrositis ossificans progressiva. J. Bone Joint Surg. 32B: 108-116.

Falk, C.C. (1954). Leukoplakia of renal pelvis and ureter. J. Urol. 72: 310-315.

Farbman, A.I. (1964). Electron microscope study of a small cytoplasmic structure in rat oral epithelium. J. Cell Biol. 21: 491-495.

Farbman, A. I. (1966). Plasma membrane changes during keratinization. Anat. Rec. 156: 269-282.

Farquhar, M.G. & Palade, G.E. (1963). Junctional complexes in various epithelia. J. Cell Biol. 17: 375-412.

Farquhar, M.G. & Palade, D.E. (1965). Cell junctions in amphibian skin. J. Cell Biol. 26: 263-291.

Farris, E.J., Yeakel, E.H. & Seltner, M.H. (1946). Ossifying cartilage and thrombi in the hearts of rats. Am. J. Path. 22: 613-619.

Fawcett, D.W. & Porter, K.R. (1954). A study of the fine structure of ciliated epithelium. J. Morphol. 94: 221-281.

Feit, J., Svejda, J. & Sochorova (1967). Experimental intestinal metaplasia of gastric mucosa of rats and its relationship to carcinoma. Neoplasma 14: 285-290.

Fell, H.B. (1957). The effect of excess vitamin A on cultures of embryonic chicken skin explanted at different stages of differentiation. Proc. Roy. Soc. B146: 242-256.

Fell, H.B. (1964). The experimental study of keratinization in organ culture. In Montagna, W. & Lobitz, W.C. ed. The epidermis. Academic Press, New York and London. 61-91.

Fell, H.B. & Dingle, J.T. (1963). Studies on the mode of action of excess of vitamin A . B. Lysosomal protease and the degradation of cartilage matrix. Biochem. J. 87: 403-408.

Fell, H.B. & Mellanby, E. (1950). Effects of Hypervitaminosis A on foetal mouse bones cultivated in vitro. Brit. Med. J. 11: 535-539.

Fell, H.G. & Mellanby, E. (1952). The effect of hypervitaminosis A on embryonic limb bones cultured in vitro. J. Physiol. 116: 320-349.

Fell, H.B. & Mellanby, E. (1953). Metaplasia produces in cultures of chick ectoderm by high vitamin A. J. Physiol. 119: 470-488.

Fell, H.B.; Mellanby, E. & Pelc, S.R. (1954). Influence of excess vitamin A on the sulphate metabolism of chick ectoderm growth in vitro. Brit. Med. J. II: 611.

Fell, H.B. & Rinaldini, L.M. (1965). The effects of vitamin A and C on cells and tissues in culture. In Willmer, E.N. ed. Cells and tissues in culture. Methods, Biology and Physiology. Academic Press, New York and London. Vol. 1: 659-699.

Fell, H.B. & Thomas, L. (1960). Comparison of the effects of papain and vitamin A on cartilage. II. The effects on organ cultures of embryonic skeletal tissue. J. Exp. Med. 111: 719-744.

Fell, H.B. & Thomas, L. (1961). The influence of hydrocortisone on the action of excess vitamin A on limb bone rudiments in culture. J. Exp. Med. 114: 343-362.

Fernando, N.V.P. & Movat, H.Z. (1963). Fibrillogenesis in regenerating tendon. Lab. Invest. 12: 214-229.

Fitton-Jackson, S.C. (1954). The formation of connective and skeletal tissues. Proc. Roy. Soc. B142: 536-548.

Fitton-Jackson, S.C. (1964). Connective tissue cells. In Brachet, J. & Mirsky, A.E. ed. The cell. Academic Press, New York Vol. VI. 387-520.

Fitton-Jackson, S.C. (1968). The Morphogenesis of collagen. In Gould, B.S. ed. Treatise on collagen: Biology of Collagen. Academic Press, London and New York, Vol.2: Part B, 1-66.

Fitton-Jackson, S. & Fell, H.B. (1963). Epidermal fine structure in embryonic chicken skin during a typical differentiation

induced by vitamin A in culture. Develop. Biol. 7: 394-419.

Foot, N.C. (1944). Glandular metaplasia of the epithelium of the urinary tract. South. Med. J. 37: 137-142.

Frasca, J.M., Auerbach, O., Parks, V.R. & Jamieson, J.D. (1968). Electron microscopic observations of the bronchial epithelium of dogs. II. Smoking dogs. Exp. Molec. Path. 9: 380-399.

Freeman, J.A. (1962). Fine structure of the goblet cell mucous secretory process. Anat. Rec. 144: 341-345.

Freeman, J.A. (1966). Goblet cell fine structure. Anat. Rec. 154: 121-148.

Frei, J.W. & Sheldon, H. (1961). A small granular component of the cytoplasm of keratinizing epithelia. J. Biophys. Biochem. Cytol. 11: 719-724.

Friedenstein, A.Y. (1959). Osteogenesis in transitional cell grafts of urinary bladder. Bull. Exp. Biol. Med. 47: 514.

Friedenstein, A.Y. (1962). Humoral nature of osteogenic activity of transitional epithelium. Nature 194: 698-699.

Friedenstein, A.Y. & Lalykina, K.S. (1963). Bone-inducing properties of transplanted transitional epithelium in rats. Bull. Exp. Biol. Med. 55: 104-107.

Friedenstein, A.Y. (1968). Induction of bone tissue by transitional epithelium. Clin. Orthopaed. 59: 21-37.

Friedenstein, A.Y., Lalykina, K.S. & Tolmacheva, A.A. (1967). Osteogenic activity of peritoneal fluid cells induced by transitional epithelium. Acta. Anat. 68: 532-549.

Frithiof, L. (1970). Ultrastructural changes in the plasma membrane in human oral epithelium. J. Ultrastruct. Res. 32: 1-17.

Frithiof, L. & Wersäll, J. (1965). A highly ordered structure in keratinizing human oral epithelium. J. Ultrastruct. Res. 12: 371-379.

Fruin, A.H., Tighe, J.R. (1967). Tubal metaplasia of the endometrium. J. Obstet. Gynaecol. Brit. Commonwealth 74: 93-97.

Fukuyama, K. & Epstein, W.L. (1969). Sulfur-containing proteins and epidermal keratinization. J. Cell Biol. 40: 830-838.

Gazayerli, M. (1964). The significance of "squamous metaplasia" as a precancerous lesion for squamous cell carcinoma on bilharzial bladders. Acta Unio. Intern. Contra Cancrum 20: 741-742.

Ghidoni, J.J. & Campbell, M.M. (1969). Fine structure of metaplastic cornified squamous epithelium in the urinary bladder of rats. J. Path. 97: 665-670.

Giacometti, L. & Montagner, W. (1967). Langerhans cells: Uptake of tritiated theymidine. Science. 157: 438

Gibbs, S.J. & Cararett, C.W. (1966). Proliferative activity in the cheek pouch epithelium of the chinese hamster. Internat. Assoc. for Dental Res. Abst. 44th Gen. Meeting, Miami, Fla. 80.

Gillette, R. (1957). Rete pegs and salivary glands of the hamster cheek pouch. 35th Gen. Meeting, Int. Assn. Dental Res. Abst. 133.

Glauert, A.M., Daniel, M.R., Lucy, J.A. & Dingle, J.T. (1963). Studies on the mode of action of excess of vitamin A. VII. Changes in the fine structure of erythrocytes during haemolysis by vitamin A. J. Cell Biol. 17: 111-121.

Godman, G.C. & Lane, N. (1964). On the site of sulfation in the chondrocyte. J. Cell Biol. 21: 353-366.

Godman, G.C. & Porter, K.R. (1960). Chondrogenesis. Studies with the electron microscope. J. Cell Biol. 8: 719-760.

Goldberg, B. & Green, H. (1964). An analysis of collagen secretion by established mouse fibroblast lines. J. Cell Biol. 22: 227-258.

Goldman, H. & Ming, Si-chun (1968). Fine structure of intestinal metaplasia and adenocarcinoma of the human stomach. Lab. Invest. 18: 203-210.

Gordon, A. (1963). Intestinal metaplasia of the urinary tract epithelium. J. Path. Bact. 85: 441-444.

Göthlin, G. & Ericsson, J.L.E. (1970). Electron microscopic studies of cytoplasmic filaments and fibers in different cell types of fracture callus in the rat. Virch. Arch. Abt. B. Zellpath. 6: 24-37.

Greig, D.M. (1933). Clinical studies in the pathology of bone. Edinb. Med. J. 40: 482-484.

Grillo, M.A. & Palay, S.L. (1963). Ciliated Schwann cells in the autonomic nervous system of the adult rat. J. Cell Biol. 16: 430-436.

Hallé, N. (1896). Cited from: Patch, F.S. (1948). Epithelial metaplasia of the urinary tract. J. Am. Med. Assoc. 136: 824-827.

Halpert, B. (1927). Morphological studies on the gall bladder. II. The "true Luschka ducts" and the Rokitansky-Aschoff sinuses of the human gall bladder. Bull. Johns Hopk. Hosp. 51: 77-103.

Halstead, A.E. & Christopher, F. (1923). Calcification and ossification of the Menings. Arch. Surg. 6: 847-857.

Ham, A. (1930). Histological study of the early phases of bone repairs. J. Bone Joint Surg. 12: 827-844.

Ham, A. (1969). Histology. 6th edition. Lippincott Co., Philadelphia and Montreal.

Handler, A.H. & Shepro, D. (1968). Cheek pouch technology: Uses and applications. In Hoffman, R.A., Robinson, P.F. and Magalhaes. H. ed. Iowa State Univ. Press, Iowa. 195-201.

Hanson, J. (1947). The histogenesis of the epidermis on the rat and mouse. J. Anat. 81: 174-196.

Hardy, M.H. (1949). The development of mouse hair *in vitro* with some observations on pigmentation. J. Anat. 83: 364-384.

Hardy, M.H. (1967). Responses in embryonic mouse skin to excess vitamin A in organotypic cultures from the trunk, upper lip and lower jaw. Exp. Cell Res. 46: 367-384.

Hardy, M.H. (1968). Glandular metaplasia of hair follicles and other responses to vitamin A excess in cultures of rodent skin. J. Embryol. Exp. Morphol. 19: 157-180.

Hartley, J. & Ianz, S.S. (1951). Experimental osteogenesis in rabbit muscle. Arch. Surg. 63: 845-851.

Hashimoto, K., Gross, B.G., Nelson, R. & Lever, W.F. (1966). The ultrastructure of the skin of human embryos. III. The formation of the nail in 16-18 week old embryos. J. Invest. Dermatol 47: 205-217.

Hashimoto, K. & Tarnowski, W.M. (1968). Some new aspects of the Langerhans Cells. Arch. Dermatol. 97: 450-464.

Hauest, M.D. & More, R.H. (1967). Electron microscopy of connective

tissues and elastogenesis. In Wagner, B.M. & Smith, D.E. ed. The connective tissue. Williams & Wilkins Co., Baltimore. 352-376.

Hayes, K.C., McCombs, H.L. & Faberty, T.P. (1970). The fine structure of vitamin A deficiency. I. Parotid duct metaplasia. Lab. Invest. 22: 81-89.

Hedenberg, I. (1954). Microscopic and microscopic change and stone formation in the urinary tract in experimentally produced vitamin A deficiency in rats. Acta Chir. Scand. Suppl. 192: 1-87.

Heinen, J.H., Dabbs, G.H. & Mason, H.A. (1949). The experimental production of ectopic cartilage and bone in the muscles of rabbits. J. Bone Joint Surg. 31A: 765-775.

Hicks, R.M. (1965). The fine structure of the transitional epithelium of rat ureter. J. Cell Biol. 26: 25-48.

Hicks, R.M. (1966). The permeability of rat transitional epithelium. Keratinization and the barrier to water. J. Cell Biol. 28: 21-31.

Hicks, R.M. (1968). Hyperplasia and cornification of the transitional epithelium in the vitamin A deficient rat. Changes in fine structure of the cells. J. Ultrastruct. Res. 22: 206-230.

Hinman, F., Kutzman, A.A. & Gibson, T.E. (1924). Leucoplakia of the kidney pelvis. With report of two cases. Surg. Gynecol. Obstet. 39: 472-489.

Hirsch, E.F. & Morgan, R.H. (1939). Causal significance to traumatic ossification of the fibrocartilage in tendon insertions. Arch. Surg. 39: 824-837.

Hisaw F. L., Meyer, R.K. & Weichert, C.K. (1928). Inhibition of ovulation and associated histological changes. Proc. Soc. Exp. Biol. Med. 25: 754-756.

Holand, (1946). Cited from: Fruin, A.H. & Tighe, J.R. (1967). Tubal metaplasia of the andometrium. J. Oststet. Gynecol. Brit. Commonwealth. 74: 93-97.

Holley, P.S., Mellinger, G.T. (1961). Leukoplakia of the bladder and carcinoma. J. Urol. 86: 235-241.

Huggins, C. B. (1930a). Transplantation of uretheral segments to the abdominal wall. Proc. Soc. Exp. Biol. Med. 28: 125-126.

Huggins, C.B. (1930b). Influence of urinary tract mucosa on the experimental formation of bone. Proc. Soc. Exp. Biol. Med. 27: 349-351.

Huggins, C.B. (1931a). The formation of bone under the influence of epithelium of the urinary tract. Arch. Surg. 22: 377-408.

Huggins, C.B. (1931b). The phosphatase activity of transplants of the epithelium of the urinary bladder to the abdominal wall producing heterotopic ossification. Biochem. J. 25: 728-732.

Huggins, C.B. & Compere, E.L. (1930). Calcium and phosphorus content of epithelial lined cysts from transplantation of mucosa of urinary bladder to rectus sheath in dogs. Proc. Soc. Exp. Biol. Med. 27: 753-755.

Huggins, C.B., McCarroll, H.R. & Blockson, B.H. (1936). Experiments on the theory of osteogenesis. Arch. Surg. 32: 913-931.

Huggins, C.B. & Sammet, J.F. (1933). Function of the gall bladder

epithelium as an osteogenic stimulus and the physiological differentation of connective tissue. J. Exp. Med. 58: 393.

Husbands, M.E. & Walker, B.E. (1963). Differentiation of vaginal epithelium in mice given estrogen and thymidine-H^3. Anat. Rec. 147: 187-198.

Ito, S. (1965). The enteric surface coat of cat intestinal microvilli. J. Cell Biol. 27: 475-491.

Järvi, O. (1944). Cited from: Jarvi, O. & Laurén, P. (1967). Intestinal metaplasia in the mucosa of the gall bladder and common bile duct, with additional observations on pancreas heterotopy. Ann. Med. Exp. Fenn. 45: 213-223.

Järvi, O. (1945). Heterotopic tumors with an intestinal mucous membrane structure in the nasal cavity. Acta Oto-laryng. (Stockh) 33: 471-485.

Järvi, O. (1962). A review of the part played by gastro-intestinal heterotopias in neoplasmogenesis. Proc. Finnish Acad. Sci. Lett. 151-187.

Järvi, O. & Laurén, P. (1951). On the role of heterotopias of the intestinal epithelium in the pathogenesis of gastric cancer. Acta Path. Microbiol. Scand. 29: 26-144.

Järvi, O. & Laurén, P. (1967). Intestinal metaplasia in the mucosa of the gall bladder and common bile duct with additional observations on pancreas heterotopy. Ann. Med. Exp. Fenn. 45: 213-223.

Järvi, O. & Meurman, L. (1964). Heterotopic gastric mucosa and pancreas in the gall-bladder with reference to the question of hetero-

topias in general. Ann. Acad. Sci. Fenn. Ser. A.5. 106/22: 1-42.

Jensen, H.M. & Mottet, N.K. (1970). Ultrastructural features of defective in vitro keratinization of chick embryonic skin. J. Cell Sci. 6: 485-509.

Jessen, H. (1970). Two types of keratohyalin granules. J. Ultrastruct. Res. 33: 95-115.

Johnson, F.R. & McMinn, R.M.H. (1956a) . Transitional epithelium and osteogenesis. J. Anat. 90: 106-116.

Johnson, F.R. & McMinn, R.M.H. (1956b). Heterotopic bone formation with autografts and homografts of bladder mucosa. Transplant. Bull. 3: 3-12.

Johnson, L.C. (1960). Mineralization of turkey leg tendons. I. Histology and histochemistry of mineralization. In calcification in biological systems. Amer. Assoc. Adv. Sci. :117- 134

Jolly, M. (1967a). Vitamin A deficiency: A review. I.J. Oral Therap. Pharmacol. 3: 365-386.

Jolly, M, (1967b). Vitamin A deficiency: A review. II. J. Oral Therap. Pharmacol. 3: 439-451.

Jones, R.W. (1932). Ossification of the Achilles tendon. Brit. Med. J. 11:943.

Kagikowa, K., Tanii, T. & Hirono. R. (1959). Electron Microscopic studies on skin fibroblasts of the mouse with special reference to the fibrillogenesis in connective tissue. Acta Path. Japon. 9: 61-67.

Kahn, R.H. (1954). Effect of locally applied vitamin A and estrogen on the rat vagina. Am. J. Anat. 95: 309-335.

Kalnins, V.I. & Porter, K.R. (1969). Centriole replication during ciliogenesis in the chick tracheal epithelium. Z. Zellforsch 100: 1-30.

Kelalis, P.P., Emmett, J.L. & DeWeerd, J.H. (1963). Leukoplakia of the urinary bladder. Report of a case with unusual features. Proc. Mayo Clinic 38: 514-518

Kerr, A.B. & Lendrum, A.C. (1936). A chloride secreting papilloma in the gall bladder. A tumour of heterotopic intestinal epithelium containing Paneth's cells and entero-chromaffine cells associated with massive chloride loss with critical review of papilloma of the gall bladder. Brit. J. Surg. 23: 615-639.

Khan, T.A. & Overton, J. (1970). Lanthanum staining of developing chick cartilage and reaggregating cartilage cells. J. Cell Biol. 44: 433-438.

Kiistalla, U. & Mustakallio, K.K. (1967). Electron microscopic evidence of synthetic activity in Langerhans cells of human epidermis. Z. Zellforsch 78: 427-440.

Kingsbury, J.W., Allen, V.G. & Rotheram, B.A. (1953). The histological structure of the beak in the chick. Anat. Rec. 116: 95-115.

Kinsky, S.C. (1963). Comparative responses of mammalian erthrocytes and microbial protoplasts to polyene antibiotics and vitamin A. Arch. Biochem. 102: 180-188.

Knee, K.H. & Biermann, H. (1958). Cited from: Scapinelli, R. & Little,K.

(1970). Observations on the mechanically induced differentiation of cartilage from fibrous connective tissue. J. Path. 101: 85-91.

Koss, L.G. (1969). The asymmetric unit membranes of the epithelium of the urinary bladder of the rat. Lab. Invest. 21: 154-168.

Kotin, P., Courington, D. & Falk, H.L. (1966). Pathogenesis of cancer in a ciliated mucous secreting epithelium. Amer. Rev. Resp. Dis. 96: Suppl. Symposium on structure function and measurement of respiratory cilia. 115-124.

Krag, D.O. & Alcott, D.L. (1957). Glandular metaplasia of renal pelvis. Am. J. Clin. Path. 27: 672-680.

Krikos, G.A. (1966). Histochemical studies of mucins of odontogenic cysts exhibiting mucous metaplasia. Arch. Oral Biol. 11: 633-640.

Krompecher, I. (1937). Cited from: Buck, R.C. (1953). Regeneration of tendon. J. Path. Bact. 66: 1-18.

Kutzmann, A.A. (1929). Leukoplakia of the renal pelvis. Arch. Surg. 19: 871-879.

Kutzmann, A.A. (1938). Squamous cell carcinoma of the renal pelvis with special consideration as to etiology. J. Urol. 39: 487-505.

Lacroix, P. (1947). Organizers and growth of bone. J. Bone Joint Surg. 29: 292-296.

Lacroix, P. (1951). The organization of bones. London: J&A Churchill Ltd.

Lasnitzki, I. (1958). The effect of carcinogens, hormones and vitamins on organ cultures. Intern. Rev. Cytol. 7: 79-121.

Lasnitzki, I. (1961). Effect of excessive vitamin A on the normal

and oestrone-treated mouse vagina grown in chemically defined medium. Exp. Cell Res. 24: 37.

Lasnitzki, I. (1962). Hypovitaminosis A in the mouse prostate gland cultured in chemically defined medium. Exp. Cell Res. 28: 40-51.

Lasnitzki, I. (1963). The effect of excess of vitamin A on the embryonic rat oesophagus in culture. J. Exp. Med. 118: 1-6.

Lasnitzki, I. & Greenberg, R. (1956). Cited from: Lasnitzki, I. (1958). The effect of carcinogens, hormones and vitamins on organ cultures. Intern. Rev. Cytol. 7: 79-121.

Lataste, F. (1892). Cited from: Meyer, R.K. & Allen, W.W. (1933). The production of mucified cells in the vaginal epithelium of certain rodents by oestrin and by corpus luteum extracts. Anat. Rec. 56: 321-343.

Lavker, R.M. & Matoltsy, A.G. (1970). Formation of horny cells. The fate of cell organelles and differentiation products in ruminal epithelium. J. Cell Biol. 44: 501-512.

Lawrence, D.J. & Bern, H.A. (1960). Mucous metaplasia and mucous gland formation in keratinized adult epithelium *in situ* treated with vitamin A. Exp. Cell Res. 21: 443-446.

Lawrence, D.J., Bern, H.A. & Steadman, M.G. (1960). Vitamin A and keratinization studies on the hamster cheek pouch. Ann. Oto. Rhinol. Laryngol. 69: 646-660.

Leeson, T.S. & Leeson, C.R. (1968). The fine structure of Bruner's glands in man. J. Anat. 103: 263-276.

Leider, M. (1950). Osteoma cutis as a result of severe acne vulgaris of long duration. Arch. Dermatol. Syph. 62: 405-407.

Leuchtenberger, C., Doolon, P.F. & Leuchtenberger, R. (1958). A correlated histological, cytological and cytochemical study of the tracheobronchial tree and lungs of mice exposed to cigarette smoke. I. Bronchitis with a typical epithelial changes in mice exposed to cigarette smoke. Cancer 2: 490-506.

Levander, G. (1938). A study of bone regeneration. Surg. Gynecol. Obstet. 67: 705-714.

Liek, E. (1906). Cited from: Huggins, C.B. (1931). The formation of bone under the influence of epithelium of the urinary tract. Arch. Surg. 22: 377-408.

Liek, E. (1908). Cited from Huggins, C.B. (1931). The formation of bone under the influence of epithelium of the urinary tract. Arch. Surg. 22: 377-408.

Lilga, H.V. & Burns, D.C. (1942). Osteomatosis cutis, report of a case. Arch. Dermatol. Syph. 46: 872-874.

Lindberg, (1935). Cited from: Niskanen, K.O. (1949). Observations on metaplasia of bronchial epithelium and its relation to carcinoma of the lung. Acta. Path. Microbiol. Scand. Suppl. 80: 1-80.

Listgarten, M.A., Albright, J.T. & Goldhaber, P. (1963). Ultra-structure alterations in hamster cheek pouch epithelium in response to carcinogen. Arch. Oral Biol. 8: 145-165.

Lloyd-Williams, I.H. (1929). On a case of bony plaques developing in the skin. Brit. Med. J. 111: 1055.

Long, J.A. & Evans, H.M. (1922). The oestrous cycle in the rat and its associated phenomena. Mem. Univ. Calif. 6:1-148.

Lucy, J.A. (1964). Membrane permeability and the control of cellular function. Natl. Cancer Inst. Monograph 13: 93-107.

Luft, J.H. (1961). Improvements in epoxy resin embedding methods. J. Biophys. Biochem. Cytol. 9: 409-414.

Luft, J.H. (1965a). The fine structure of hyaline matrix following ruthenium red fixative and staining. J. Cell Biol. 27: 61A.

Luft, J.H. (1956b). Ruthenium red and violet. I. Chemistry purification, methods of use and mechanism of action. Preprint

MacDonald, A.M. & Robson, J.M. (1939). The production of vaginal mucification with the synthetic oestrogen, tri-phenyl ethylene. J. Path. Bact. 48: 95-98.

McLoughlin, C.B. (1961a). The importance of mesenchymal factors in the differentiation of chick epidermis. I. The differentiation in culture of the isolated epidermis of the embryonic chick and its response to excess vitamin A. J. Embryol. Exp. Morphol. 9: 370-384.

McLoughlin, C.B. (1961b). The importance of mesenchymal factors in the differentiation of chick epidermis. II. Modification of epidermal differentiation by contact with different types of mesenchyme. J. Embryol. Exp. Morph. 9: 385-409.

Madsen, P.O., Slotkin, E.A., Niesen, W.F. (1961). Leukoplakia of the renal pelvis with review of the literature. Acta. Chir. Scand. 121: 47-55.

Makin, M. (1962). Osteogenesis induced by vesical mucosal transplant in the guinea-pig. J. Bone Joint Surg. 44B: 165-193.

Mallinson, F.B. (1932). Ossification of both Achilles tendons with traumatic fracture of one. Brit. Med. J. 11: 836-837.

Manasek, F.J. (1968). Mitosis in developing cardiac muscle. J. Cell Biol. 37: 191-196.

Marshall, V.F. & Spellman, R.M. (1957). Free grafts of mucosa from the urinary bladder. I. For construction of a urethra in human. II. For production of bone in dogs. Plastic and Reconstructive Surg. 20: 423-436.

Martin, J.H. & Matthews, J.L. (1969). Mitochondrial granules in chondrocytes. Calc. Tiss. Res. 3: 184-193.

Masson, P. (1951). My concept of cellular nevi. Cancer 4: 9-38.

Matoltsy, A.G. (1966). Membrane-coating granules of the epidermis. J. Ultrastruct. Res. 15: 510-515.

Matoltsy, A.G. (1969). Keratinization of the avian epidermis. An ultrastructural study of the newborn chick skin. J. Ultrastruct. Res. 29: 438-458.

Matoltsy, A.G. & Parakkal, P.F. (1965). Membrane coating granules of keratinizing epithelia. J. Cell Biol. 24: 297-307.

Matoltsy, A.G. & Parakkal. P.F. (1967). Keratinization. In Zelickson, A. ed. Ultrastructure of normal and abnormal skin. Lee and Febiger, Philadelphia. 76-104.

Matthews, J.L. (1970). Ultrastructure of calcifying tissues. Am. J. Anat. 129: 451-458.

Matthews, L.J., Martin, J.H., Lynn, L.A. & Collins, E.J. (1968). Calcium incorporation in the developing cartilaginous epiphysis. Calc. Tiss. Res. 1: 330-336.

Matukas, V.J., Panner, B.J. & Orbison, J.L. (1966). Studies on ultrastructural indentification and distribution of protein-polysaccharide in cartilage matrix. J. Cell Biol. 32: 365-378.

Maudsley, R.H. (1952). A case of myositis ossificans progressiva. Brit. Med. J. 1: 954-956.

Mercer, E.H. (1961). Keratin and keratinization. Pergamen Press, New York.

Mestel. A.L. & Spain, D.M. (1967). Differences in bone formation induced by urinary bladder autografts, homografts and pedicle grafts. Exp. Molec. Path. 6: 118-130.

Meyer, R.K. & Allen, W.M. (1933). The production of mucified cells in the vaginal epithelium of certain rodents by oestrin and by corpus luteum extracts. Anat. Rec. 56: 321-343.

Meyrick, B. & Reid, L. (1970). Ultrastructure of cells in the human bronchial submocosal glands. J. Anat. 107: 281-299.

Milhaud, M. & Pappas, G.D. (1968). Cilia formation in the adult cat brain after pargyline treatment. J. Cell Biol. 37: 599-609.

Ming, S.C., Goldman, H. & Freiman, D.G. (1967). Intestinal metaplasia and histogenesis of carcinoma in human stomach. Light and electron microscopic study. Cancer 20: 1418-29.

Miszurski, B. (1937). Researches on the keratinization of the epithelium in tissue culture. Arch. Exp. Zellforsch 20: 122-139.

Monis, B. & Dorfman, H.D. (1967). Some histochemical observations on transitional epithelium of man. J. Histochem. Cytochem. 15: 475-481.

Monis, B. & Zambrano, D. (1968a). Transitional epithelium of urinary tract in normal and dehydrated rats. A histochemical and electron microscopic study. Z. Zellforsch 85: 165-182.

Monis, B. & Zambrano, D. (1968b). Ultrastructure of transitional epithelium of man. Z. Zellforsch 87: 101-117.

Montagna, W. (1962). The structure and function of skin. 2nd ed. Academic Press, New York.

Moore, T. (1957). Vitamin A. Amsterdam, Elesevier.

Mori , S. (1922). The changes in the para-occular glands which follow the administration of diets low in fat-soluble A; with notes on the effect of the same diets on the salivary glands and the mocusa of the larynx and trachea. Bull, John Hopkins Hosp. 33: 357-359.

Moscona, A., Trowell, O.A. & Willmer, E.N. (1965). Methods. In Willmer, E.N. ed. Cell and Tissues in Cultures. Methods, Biology and physiology. Academic Press, London, New York, Vol. 1: 19-98.

Mostofi, F.K. (1954). Potentialities of bladder epithelium. J. Urol. 71: 705-714.

Mostofi, F.K., Thompson, R.V. & Dean, A.L. Jr. (1955). Mucous adenocarcinoma of the urinary bladder. Cancer 8: 741-758.

Mottet, N.K. & Jensen, H.M. (1968). The differentiation of chick embryonic skin. Exp. Cell Res. 52: 261-283

Movat, H.Z. & Fernando, N.V.P. (1962). The fine structure of connective tissue. I. The fibroblast. Exp. Molec. Path. 1: 509-534.

Nachinias, V.T. (1964). Fibrillar structures in the cytoplasm of Chaos chaos. J. Cell Biol. 23: 183-188.

Neuhof, H. (1917). Fascia transplantation into visceral defects. Surg. Gynecol. Obstet. 24: 383-427.

New, D.A.T. (1963). Effects of excess vitamin A on cultures of skin and buccal epithelium of the embryonic rat and mouse. Brit. J. Dermatol 75: 320-325.

New, D.A.T. (1965). Effects of excess vitamin A on cultures of skin from the tail and pads of the embryonic rat and from the trunk, tail and pads of the embryonic rabbit. Exp. Cell Res. 39: 178-183.

Nicholson, G.W. (1917). The formation of bone in a calcified epithelium of the skin with some remarks on metaplasia. J. Path. Bact. 21: 287-304.

Nicholson, G.W. (1922a). The heteromorphoses in the human body. Guy's Hosp. Rep. 72: 75-127.

Nicholson, G.W. (1922b). Studies on tumour formation. Acquired tissue malformation. Guy's Hosp. Rep. 72: 402-422.

Niskanen, K.C. (1949). Observations on metaplasia of bronchial epithelium and its relation to carcinoma of the lung. Acta Path. Microbiol. Scand. Suppl. 80: 1-80.

Novak, E. (1932). The morphology of the genital epithelia with special reference to differentiaion anomolies. Am. J. Obstet. Gynecol. 24: 635-653.

Odland, G.F. (1958). The fine structure of the interrelationship of cells in the human epidermis. J. Biophys. Biochem. Cytol. 4: 529-538.

Odland, G.F. (1960). A submiscroscopic granular component in human epidermis. J. Invest. Dermatol 34: 11-15.

Odland, G.F. & Reed, T.H. (1967). Epidermis. In Zelickson, A.S. ed. Ultrastructure of Normal and Abnormal Skin. Lee and Febiger, Philadelphia. 54-75.

O'Flynn, J.D. & Mullaney, J. (1967). Leukoplakia of the bladder. A report on 20 cases, including two cases progressing to squamous cell carcinoma. Brit. J. Urol. 39: 461-471.

of the pelvic epithellum and heteroplastic bone formation. J. Med. Res. 20: 53-66.

Peer, L.A. (1955). Transplantation of tissues. Williams & Wilkins Co., Baltimore, P. 102.

Phemister, P. B. (1923). Ossification in kidney stones attached to the renal pelvis. Ann. Surg. 78: 239-249.

Politano, V.A. (1956). Leukoplakia of the renal pelvis and ureter. J. Urol. 75: 633-642.

Pollard, I., Martin, L. & Shorey, C.D. (1966). The effect of intravaginal estradial 3:17β on the cell structure of the vaginal epithelium of the ovariectomized mouse. Steroids 8: 805-823.

Porter, K.R. (1961). The ground substance; Observations from electron microscopy. In Brachet, J. & Mirsky, A.E, ed. The Cell. Academic Press, New York, Vol 11: 621-675.

Porter, K.R., Kenyon, K. & Badenhausen, S. (1967). Specialization of the unit membrane. Protoplasma 63: 262-274.

Prunieras, M. (1969). Interactions on cytoplasmic organelles in Langerhans cells of human epidermis. J. Invest. Dermatol 52: 1-17.

Rabson, S.M. (1936). Leukoplakia and carcinoma of the urinary bladder. Report on a case with review of the literature. J. Urol. 35: 321-341.

Rash, J.E., Shay, J.W. & Biesele, J.J. (1969). Cilia in cardiac differentiation. J. Ultrastruct Res. 29: 470-484.

Reingold, I.M., Ottoman, R.E. & Konwaler, B.E. (1950). Bronchogenic Carcinoma: A study of 60 necropsies. Am. J. Clin. Path. 20: 515-525.

Olson, J.A. (1969a). Some aspects of vitamin A metabolism. Vitamin and Hormones 26: 1-63.

Olson, J.A. (1969b). Recent developments in the fat-soluble vitamins. Metabolism and function of vitamin A. Federation Proc. 28: 1670-1677.

Palay, S.L. (1961). Structural peculiarities of the neurosecretory cells in the preoptic nucleus of the goldfish Carassius auratus. Anat. Rec. 139: 262.

Papanicolaou, G.N. & Koprowska, I. (1951). Carcinoma in situ of the right lower bronchus: A case report. Cancer 4: 141-146.

Parakkal, P.F. (1967). An electron microscopic study of esophageal epithelium in the newborn and adult mouse. Am. J. Anat. 121: 175-196.

Parakkal, P.F. & Matoltsy, A.G. (1968). An electron microscopic study of developing chick skin. J. Ultrastruct. Res. 23: 403-416.

Pasternak, C.A., Humphries, S.K. & Pirie, A. (1963). The activation of sulphate by extracts of cornea and colonic mucosa from normal and vitamin A deficient animals. Biochem. J. 86: 382-4.

Patch, F.S. (1948). Epithelial metaplasia of urinary tract. J. Am. Med. Assoc. 136: 824-827.

Patch, F.S. & Rhea, L.J. (1935). The genesis and development of Brun's nests and their relation to cystitis cystica, cystitis glandularis and primary adenocarcinoma of the bladder. Can. Med. Assoc. J. 3: 597-612.

Pearce, R.M. (1909). Notes of the later stages of the repair of kidney tissue (dog) with special reference to proliferation

Retterer, E. (1892). Cited from: Meyer, R.K. & Allen, W.W. (1933). The production of mucified cells in the vaginal epithelium of certain rodents by oestrin and by corpus luteum extracts. Anat. Rec. 56: 321-343.

Revel, J.P. & Hay, E.D. (1963). An antoradiographic and electron microscopic study of collagen synthesis in differentiating cartilage. Z. Zellfrosch. 61: 110-144.

Reynolds, E.S. (1963). The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J. Cell Biol. 17: 208-212.

Rhodin, J. (1959). Ultrastructures of the ciliated mucosa in rat and man. Ann. Otol. Rhinol. Laryngeol 68: 964-974.

Rhodin, J. (1966). Ultrastructure and function of the human tracheal mucosa. Am. Rev. Resp. Dis. Suppl. 93: 1-15.

Rhodin, J. & Dalhamn, T. (1956). Electron microscopy of the tracheal ciliated mucosa in rat. Z. Zellforsch 44: 345-412.

Rhodin, J.A.G. & Reith, E.J. (1962). Ultrastructure of keratin of oral mucosa, skin, esophagus, claw and hair. In Butcher, E.D. & Sognnaes, R.F. ed. Fundamentals of keratinization. Am. Assoc. Advance Sci., Washington, D.C. Publ. 70: 61-94.

Rigdon, R.H. (1959). Effect of Methylcholanthrene on the respiratory tract of the white Pekin duck. Arch. Path. 68: 578-588.

Robinson, R.A. & Cameron, D.A. (1956). Electron microscopy of cartilage and bone matrix at the distal epiphyseal line of the femur in the newborn infant. J. Biophys. Biochem. Cytol. 2: 253-274.

Robson, J.M. & Weisner, B.P. (1931). Causation of mucification and cornification in the vagina of the mouse. Quart. J. Exp. Physiol 21: 217-225.

Rockey, E.E., Speer, E.D., Ahn, K.J., Thompson, S.A. & Hirne, T. (1962). The effect of cigarette smoke condensate on the bronchial mucosa of dogs. Cancer 15: 1100-1116.

Rockey, E.E. & Speer, F.D. (1966). The ill effects of cigarette smoking in dogs. Intern. Surg. 46: 520-530.

Rockey, E.E., Kuschner, M., Kosak, A.I. & Meyer, E. (1958). The effect of tobacco tar on the bronchial mucosa of dogs. Cancer 11: 466-742.

Roels, O.A., Trout, M. & Guha, A. (1964). Vitamin deficiency and acid hydrolasses: β-ylycerophosphate phosphatase in rat liver. Biochem. J. 93: 230-250.

Rogers, G.E. (1964). Structural and biochemical features of the hair follicle. In Montagna, W. & Lobitz, W.C. ed. The epidermis. Academic Press, New York, P. 179-236.

Ross, R. (1968). The connective tissue fiber forming cell. In Gould, B.S. ed. Treatise on collagen. Biology of Collagen. Academic Press, London and New York. Vol. 2: 1-82.

Ross, R. & Benditt, E.P. (1961). Wound healing and collagen formation. 1. Sequential changes in components of guinea pig skin wounds observed in electron microscope. J. Biophys. Biochem. Cytol. 11: 677-700.

Ross, R. & Benditt, E.P. (1965). Wound healing and collagen formation. V. Quantitative electron microscopic radioautographic observation of proline-^{3} utilization by fibroblasts. J. Cell Biol. 27: 83-106.

Ross, J.J. & Jones, M.A. (1967). The [illegible] and survival activity of the bee [illegible] (Apis mellifera [illegible])

skin during the resting phase. J. Ultrastruct. Res. 18: 304-323.

Roussakoff, A.V. (1959). To the problem of phylogenesis of osseous matter. In Vinogradova, T.P. ed. Pathological Anatomy of Disease of Osseous System. Medgis, Moscow. 19-26.

Rowden, G. (1966). Membrane-coating granules of mouse esophageal and gastric epithelium. J. Invest. Dermatol 47: 359-362.

Sacerdotti, C. & Frattin, G. (1902). Cited from: Huggins, C.B. (1931). The formation of bone under the influence of epithelium of the urinary tract. Arch. Surg. 22: 377-408.

Sagebiel, R.W. & Reed, T.H. (1968). Serial reconstruction of the characteristic granule of the Langerhans cell. J. Cell Biol. 36: 595-602.

Salpeter, M.M. (1968). H^3-proline incorporation into cartilage: Electron microscope autoradiographic observations. J. Morphol. 124: 387-422.

Saphir, O. & Kurland, S.K. (1939). Adenocarcinoma of the urinary bladder. Urol. Cutan. Rev. 43: 709-719.

Scapinelli, R. (1960a). Cited from: Scapinelli, R. & Little, K. (1970). Observations on the mechanically induced differentiation of cartilage from fibrous connective tissue. J. Path. 101: 85-91.

Scapinelli, R. (1960b). Cited from: Scapinelli, R. & Little, K. (1970). Observations on the mechanically induced differentiation of cartilage from fibrous connective tissue. J. Path. 101:85-91.

Scapinelli, R. (1963). Sesamoid bones in the ligamentum nuchae of man. J. Anat. 97: 417-422.

Scapinelli, R. & Little, K. (1970). Observations on the mechanically induced differentiation of cartilage from fibrous connective tissue. J. Path. 101: 85-91.

Schabad. A.L. (1959). Is leucoplakia to be regarded as precarcinomatous. Z. Urol. 52: 520-525.

Schellander, F. & Wolff, K. (1967). Zur autoradiographischen merkierung von Langerhands-Zellen mit ^{3}H-thymidin. Arch. Klin. Exp. Dermatol 230: 140-151.

Scherft, J.P. (1968). The ultrastructure of the organic matrix of calcified cartilage and bone in embryonic mouse radii. J. Ultrastruct. Res. 23: 333-343.

Scherft, J.P. & Daems, W.T.H. (1967). Single cilia in chondrocytes. J. Ultrastruct. Res. 19: 546-555.

Scholl, A.J. (1922). The potential malignancy in exstrophy of the bladder. Ann. Surg. 75: 365-371.

Schulze, W.C. (1929). Cited from: Willis, R.A. (1958). The borderland of embryology and pathology. Butterworth, London.

Schuster, F.L. (1964). Ciliated fibroblasts from a human brain tumour. Anat. Rec. 150: 417-421.

Seifried, O. (1930). Studies on A-avitaminosis in chickens I. Lesions of the respiratory tract and their relation to some infectious diseases. J. Exp. Med. 52: 519-532.

Selle, R.M. (1922). Changes in the vaginal epithelium of the guinea-pig during the oestrus cycle. Am. J. Anat. 30: 429-449.

Selye, H. (1940). Effect of chronic progesterone overdose on the female accessory sex organs of normal ovariectomized and hypophysectomized rats. Anat. Rec. 78: 253-271.

Shackleford, J.M. & Wilborn, W.H. (1970a). Ultrastructural aspects of cat submandibular glands. J. Morph. 131: 253-276.

Shackleford, J.M. & Wilborn, W.H. (1970b). Ultrastructural aspects of calf submandibular glands. Am. J. Anat. 127: 259-280.

Shapiro, I.M. & Greenspan, J.S. (1969). Are mitochondria directly involved in biological mineralization? Calc. Tiss. Res. 3: 100-102.

Sheldon, H. (1964). Cartilage. In Kurtz, S.M. ed. Electron microscopic anatomy. Academic Press, New York and London, 295-313.

Simon, R. (1967). The cultivation of embryonic chicken skin in a chemicaliy defined medium and the response of the epidermis to excess of vitamin A. I. Invest. Dermatol. 49: 35-38.

Sorokin, S.P. (1962). Centrioles and the formation of rudimentary cilia by fibroblasts and smooth muscle cells. J. Cell Biol. 15: 363-377.

Sorokin, S.P. (1968). Reconstructions of centriole formation and ciliogenesis in mammalian lungs. J. Cell Sci. 3: 207-230.

Spain, D.M. (1959). The distinction between regeneration and atypical alterations in the bronchial mucosa. Am. Rev. Tuberc. 79: 591-596.

Spearman, R.I.C. (1964). The evolution of mammalian keratinized structures. In Ebling, F.J. ed. The mammalian epidermis and its derivative. Zool. Soc. Symp. 12: 67-81.

Spearman, R.I.C. (1966). The keratinization of epidermal scales, feathers and hairs. Biol. Rev. 41: 59-96.

Spouge, J.D. (1966). Sebaceous metaplasia in the oral cavity occurring in association with dentigerous cyst epithelium. Report of a

case. Oral Surg. Oral Med. Oral Path. 21: 492-498.

Stirling, W.C. & Ash, J.E. (1944). Chronic proliferation lesions of the urinary tract. J. Urol. 415: 342-360.

Strassberg, M. (1911). Cited from: Willis, R.A. (1958). The borderland of embryology and pathology. Butterworth, London.

Straub, M. & Mulder, J. (1948). Epithelial lesions in the respiratory tract in human influenzal pneumonia. J. Path. Bact. 60: 429-434.

Strauss, A.A. (1914). An artificial ureter made from the abdominal wall. A preliminary report of an experimental study. Surg. Gynecol. Obstet. 18: 78-84.

Striker, T.W., Stool, S., & Downes, J.J. (1967). Prolonged nasatracheal intubation in infants and children. Arch. Otolaryngol 85: 210-213.

Stubblefield, E. & Brinkley, B.R. (1966). Cilia formation in Chinese hamster fibroblasts in vitro as a response to Colcemid treatment. J. Cell Biol. 30: 645-652.

Svejda, J. & Dolezel, S. (1964). Bronchial precanceroses and their relationship to the cytodiagnosis of bronchial cancer. Acta U.I.C.C. 20: 740.

Symchych, P.S. & Cadott, M. (1967). Squamous metaplasia and necrosis of the trachea complicating prolonged nasatracheal intubation of small new born infants. An autopsy study. J. Pediat. 71: 534-541.

Takuma, S. (1960). Electron microscopy of the developing cartilaginous epiphysis. Arch. Oral Biol. 2: 111-119.

Tarnowski, W.M. & Hashimoto, K. (1967). Langerhans cell granules in histiocytosis X. Arch. Dermatol. 96: 298-304.

Taxi, J. (1961). Cited from: Milhaud, M. & Pappas, G.D. (1968). Cilia formation in the adult cat brain after pargyline treatment. J. Cell Biol. 37: 599-609.

Taylor, A.C. (1966). Microtubules in the microspikes and cortical cytoplasm of isolated cells. J. Cell Biol. 28: 155-168.

Teplitz, C., Epstein, B.S., Rose, L.R. & Moncrief, J.A. (1964). Necrotizing tracheitis induced by tracheostomy tube. Arch. Path. 77: 6-19.

Teutschlaender, O. (1919). Cited from: Niskanen, K.O. (1949). Observations on metaplasia of the bronchial epithelium and its relation to carcinoma of the lung. Acta Path. Microbiol. Scand. Suppl. 80: 1-73.

Thomas, R.C. (1913). Cited from: Buck, R.C. (1953). Regeneration of tendon. J. Path. Bact. 66: 1-18.

Thomas, L., McCluskey, R.T., Potter, J.L. & Weissman, G. (1960). Comparison of the effects of papain and vitamin A on cartilage. I. The effects in rabbits. J. Exp. Med. 111: 705-718.

Thompson, G.J. & Stein, J.J. (1944). Leukoplakia of the bladder and a report of 34 cases. J. Urol. 44: 639-649.

Thorsness, E.T. (1941). The relationship of "true Luschka ducts", adenomas and abberrant liver tissue in the wall of the human gall bladder. Am. J. Clin. Path. 11: 878-881.

Thyberg, J. & Friberg, U. (1970). Ultrastructure and acid phosphatase activity of matrix vesicles and cytoplasmic dense

bodies in the epiphyseal plate. J. Ultrastruct. Res. 33: 554-573.

Tipton, D.L. & Crocker, T.T. (1964). Duration of bronchial squamous metaplasia produced in dogs by cigarette smoke condensate. J. Natl. Can. Inst. 33: 487-495.

Trier, J.C. & Rubin, C.E. (1965). Electron microscopy of the small intestine: A review. Gastro-enterology 49: 574-603.

Trueta, J. (1963). The role of the vessels in osteogenesis. J. Bone Joint Surg. 45B: 402-418.

Tyson, M.D. & Smith, A.H. (1929). Tissue changes associated with vitamin A deficiency in the rat. Am. J. Path. 5: 57-69.

Umiker, W. & Storey, C. (1952). Bronchogenic carcinoma in situ: report of a case with positive biopsy, cytological examination and labectomy. Cancer 5: 369-374.

Urist, M.R. & McLean, F.C. (1952). Osteogenetic potency and new-bone formation by induction in transplants to the anterior chamber of the eye. J. Bone Joint Surg. 34A: 443-470.

Valentine, E.H. (1957). Squamous metaplasia of the bronchus. Cancer 10: 272-279.

Vero, F.; Machacek, G.F. & Bartlett, F.H. (1945). Disseminated congenital osteomas of the skin with subsequent development of myositis ossificans. Report of a case in an infant. J. Am. Med. Assoc. 129: 728-734.

Voelz, H. (1964). The "spindle-shaped body" in fibroblasts. J. Cell Biol. 20: 333-337.

Watson, M.L. (1958). Staining of tissue sections for electron microscopy with heavy metals. J. Biophys. Biochem. Cytol. 4: 475-478.

Weinstock, M. & Wilgram, G.F. (1970). Fine structural observations on the formation and enzymatic activity of keratinosomes in mouse tongue filiform papillae. J. Ultrastruct. Res. 30: 262-274.

Weiss, P. & James, R. (1955). Skin metaplasia *in vitro* induced by brief exposure to vitamin A. Exp. Cell Res. Suppl. 3: 381-384.

Welcker, E.R. (1950). Cited from: Willis, R.A. (1958). The borderland of embryology and pathology. Butterworths Med. Publ. London.

Weller, R.W. (1953). Metaplasia of bronchial epithelium: a post-mortem study. Am. J. Clin. Path. 23: 768-535.

Welsh, R.A. (1966). Intracytoplasmic collagen formations in desmoid fibromatosis. J. Path. 49: 515-535.

Wessells, N.K. (1961). An analysis of chick epidermal differentiation *in situ* and *in vitro* in chemically defined media. Develop. Biol. 3: 355-538.

Wessells, N.K. (1962). Tissue interaction during skin histo-differentiation. Develop. Biol. 4: 87-107.

Wheeler, H.R. & Mohuiddin, A. (1968). Metaplastic bone. J. Anat. 103: 527-538.

Wiesner, B.P. & Patel, J.S. (1929). The Beta Hormone. Nature 123: 449.

Wilgram, G.F. & Weinstock, A. (1966). Advances in genetric dermatology. Dyskeratosis, Acantholysis, and Hyperkeratosis, with a note on the specific role of desmosomes and keratinosomes in the formation of horny layer. Arch. Dermatol. 94: 456-479.

Wilhelm, D.L. (1953). Regeneration of tracheal epithelium. J. Path. Bact. 65: 543-550.

Williams, M.J. (1952). Extensive carcinoma *in situ* in bronchial mucosa associated with two invasive bronchogenic carcinomas. Cancer 5: 740-747.

Willis, R.A. (1958). The borderland of Embryology and Pathology. Butterworth, London.

Wlodarski, K., Hinek, A. & Ostrowski, K. (1970). Investigations on cartilage and bone induction in mice grafted with FL and WISH line human amniotic cells. Calc. Tiss. Res. 5: 70-79.

Wolbach, S.B. (1937). The pathologic changes resulting from vitamin deficiency. J.A.M.A. 108: 7-13.

Wolbach, S.B. & Howe, P.R. (1925). Tissue changes following deprivation of fat-soluble A vitamin. J. Exp. Med. 42: 753-778.

Wolbach, S.B. & Howe, P.R. (1933). Epithelial repair in recovery from vitamin A deficiency. An experimental study. J. Exp. Med. 57: 511-526.

Wolf, G. (1962). Some thoughts on the metabolic role of vitamin A. Nutr. Rev. 20: 161-163.

Wolf, G., Bergan, J.G. & Sundaresan, P.R. (1963). Vitamin A and mucopolysaccharide biosynthesis by cell-free particle suspensions. Biochem. Biophys. Acta 69: 524-532.

Wolf, G. & Varandani, P.T. (1960). Studies on the function of vitamin A in mucopolysaccharide biosynthesis. Biochem. Biophys. Acta 43: 501-512.

Wolf, G., Varandani, P.T. & Johnson, B.C. (1961). Vitamin A and mucopolysaccharide synthesizing enzymes. Biochem. Biophys. Acta 46: 59-67.

Wolff, K. (1967). The fine structure of the Langerhans cell granule. J. Cell Biol. 35: 468-473.

Wolff, K. & Holubar, K. (1967). Cited from: Bonneville, M.A., Weinstock, M. & Wilgram, G.F. (1968). An electron microscopic study of cell adhesion in psoriatic epidermis. J. Ultrastruct. Res. 23: 15-43.

Wolff, K. & Schreiner, E. (1970a). Epidermal lysosomes. Electron microscopic-cytochemical studies. Arch. Derm. 101: 276-286.

Wolff, K. & Schreiner, E. (1970b). Uptake, intracellular transport and degradation of exogenous protein by Langerhans cells. An electron microscopic-cytochemical study using peroxidase as tracer substance. J. Invest. Dermatol 54: 37-47.

Wolff, K. & Sollereder, B. (1969). Intranuclear and extracellular Langerhans cell granules. J. Invest. Dermatol 52: 424-429.

Womack, N.A. & Graham, E.A. (1942). Developmental abnormalities of the lung and bronchiogenic carcinoma. Arch. Path. 34: 301-318.

Yardley, J.H. (1962). Specialized functions of connective tissue cells. In Conference on the Biology of connective tissue. New York Arthritis and Rheumatism Foundation. 179-192.

Yardley, J.H., Heaton, M.W., Gaines, L.M. Jr. & Shulman, L.E. (1960). Collagen formation by fibroblasts. Bull, John Hopkins Hosp. 106: 381-396.

Zachman, R.D. (1967). The stimulation of RNA synthesis *in vivo* and *in vitro* by retinol (vitamin A) in the intestine of vitamin A deficient rats. Life Sci. 6: 2207-2213.

Zelickson, A.S. (1965). The Langerhans cell. J. Invest. Dermatol 44: 201-212.

Zelickson, A.S. (1966). Granule formation in the Langerhans cell. J. Invest. Dermatol 47: 498-502.

Zelickson, A.S. (1967). Melanocyte, melanin granules and Langerhans cell. In Zelickson, A.S. ed. Ultrastructure of Normal and Abnormal Skin. Lea and Febriger, Philadelphia, 163-182.

Zelickson, A.S. & Hartmann, J.F. (1961). The fine structure of the melanocyte and melanin granules. J. Invest. Dermatol 36: 23-27.